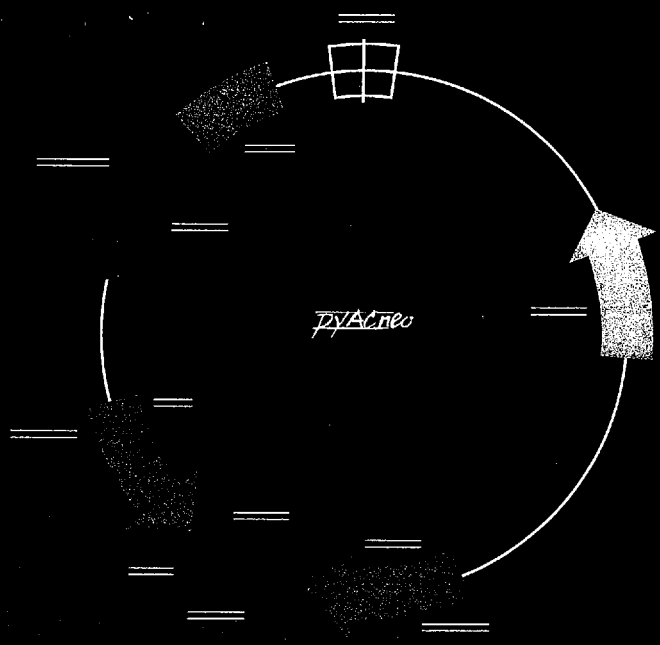
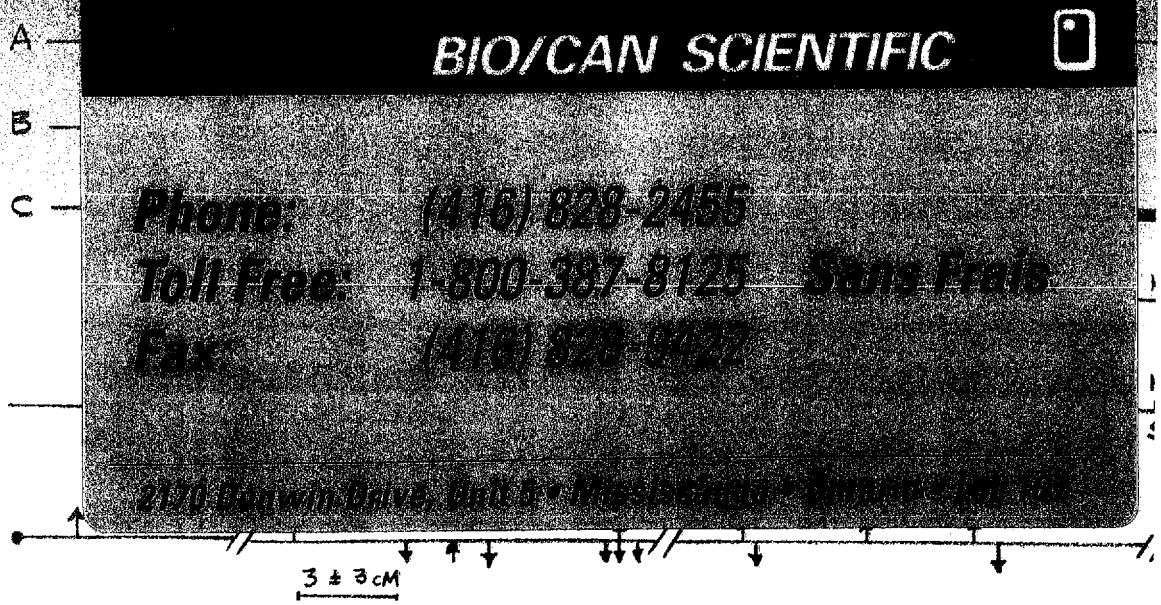
# C L O N T E C H

TOOLS FOR THE MOLECULAR BIOLOGIST 91/92









## PROKARYOTIC EXPRESSION VECTORS

# pPROK-1

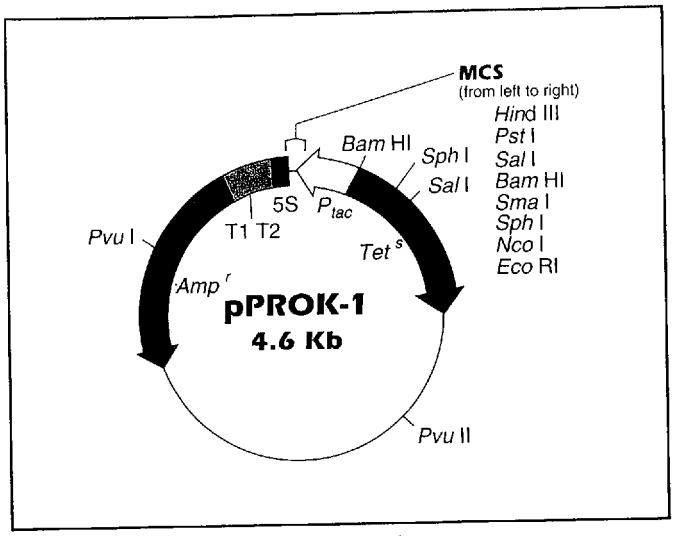



**Figure 12.1** Expression vector pPROK-1

# pPROK-C

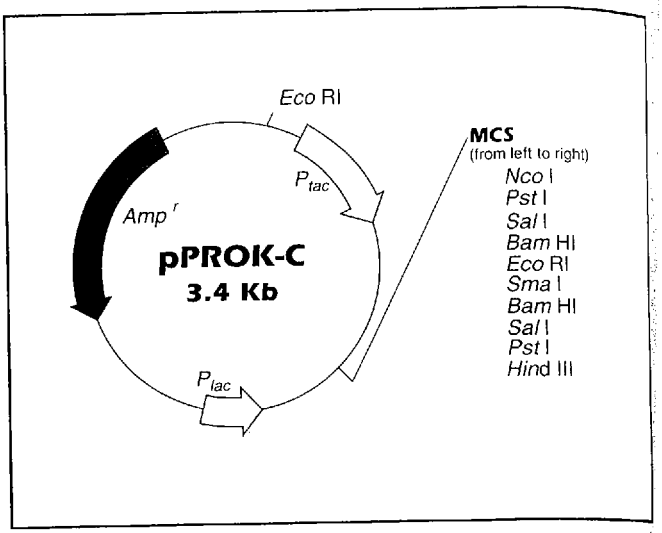



**Figure 12.2** Expression vector pPROK-C

12

**MCS**

-35
5'...TGT TGA CAA TTA ATC ATC GGC TCG TAT AAT GTG TGG AAT TGT GAG CGG

RBS
ATA ACA ATT TCA CAC AGG AAA CAG AAT TCA
Eco RI

Sph I Bam HI Pst I
TGC CAT GGC ATG CCC GGG GAT CCG TCG ACC TGC AGC CAA GCT T...3'
Nco I Sma I Sal I Hind III

**MCS**

Nco I Sal I
CCATGGCTGCAGGTCGACGGATCC
Pst I Bam HI

Eco RI Bam HI Pst I
GGGAATTCCCGGGGATCCGTCGACCTGCAGCCAAGCTT
Sma I Sal I Hind III

| Product | Size | Cat. # |
|---|---|---|
| pPROK-1 | 25 µg | 6002-1 |

| Product | Size | Cat. # |
|---|---|---|
| pPROK-C | 10 µg | 6000-1 |

### Description

The prokaryotic expression vector pPROK-1 contains *tac*, a strong promoter capable of induction by IPTG in *E. coli* strain JM109. Downstream of the Multiple Cloning Site (MCS) is the strong T1 T2 terminator from the *E. coli rrnB* gene preventing unstable replication thus allowing efficient transcript termination. The plasmid mediates both ampicillin and tetracycline resistance.

pPROK-1 has a ribosomal binding site and an ATG start codon positioned immediately upstream of the cloning site for expression of gene fragments which lack these functional units.

### Unique cloning sites within the MCS region

*Eco* RI, *Nco* I, *Sma* I, *Pst* I, *Hind* III

### Vector Size

4.6 Kb

### References


1. Brosius, J. (1984) *Gene* **27**:151.
2. Brosius, J. (1984) *Gene* **27**:161.
3. Amann, E. & Brosius, J. (1985) *Gene* **40**:183.


### Description

pPROK-C is a high efficiency prokaryotic cDNA expressio vector. pPROK-C is especially suited for the construction cDNA expression libraries. These libraries may be screene with both antibody and synthetic oligomer probes. pPRO C can be used in a wide range of *E. coli* hosts. This vect contains diametrically opposed *lacZ* and *tac* promote on either side of the Multiple Cloning Site (MCS). Bo strands of the inserted DNA can be transcribed a translated, thus cDNA inserted into this vector has tw the probability of being expressed. pPROK-C carries own ribosomal binding site and initiation ATG st codon.

### Unique cloning sites within the MCS region

*Sma* I, *Nco* I, *Hind* III

### Vector Size

3.4

# pEX1, pEX2, pEX3

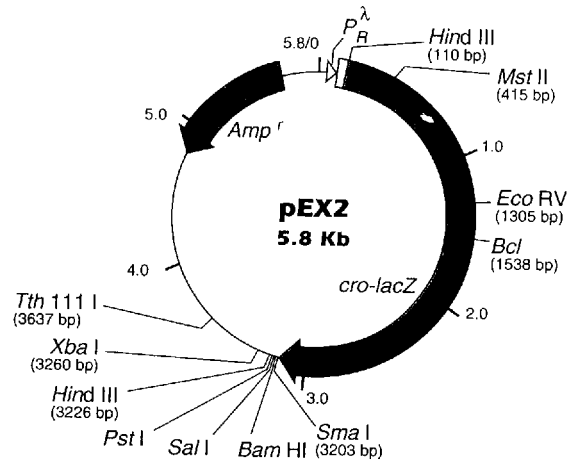



**Figure 12.3** Expression vector pEX 2

## pEX plasmids polylinker sequences

```
                Sma I  Bam HI     Sal I              Pst I   HindIII
                  |      |         |                  |       |
pEX1    GCC CGG GGA TCC GTC GAC CTG CAG CCA AGC TTG CTG ATT GAT TGA
          Ala Arg Gly Ser Val Asp Leu Gln Pro Ser Leu Leu Ile Asp  •

        Eco RI          Sma I  Bam HI     Sal I              Pst I   Hind III
          |               |      |         |                  |       |
pEX2    GAA TTC CCG GGG ATC CGT CGA CCT GCA GCC AAG CTT GCT GAT TGA
          Glu Phe Pro Gly Ile Arg Arg Pro Ala Ala Lys Leu Ala Asp  •

                        Sma I  Bam HI     Sal I              Pst I   Hind III
                          |      |         |                  |       |
pEX3    GAA TTA ATT CCC GGG GAT CCG TCG ACC TGC AGC CAA GCT TGC TGA
          Glu Leu Ile Pro Gly Asp Pro Ser Thr Cys Ser Gln Ala Cys  •
```

### Description

The pEX family of vectors allow expression of β-galactosidase fusion proteins in *E. coli*. Thermo-inducible expression is driven by the lambda $P_R$ promoter when grown in a suitable *E. coli* host strain carrying the temperature-sensitive repressor cI857(*E. coli* strain N4830-1). The lambda cro gene supplies the ATG start codon. Easy purification of β-galactosidase fusion proteins may be performed on an immunoaffinity column. The *Eco* RI site of pEX2 was 1) treated with SI nuclease to create pEX1, and 2) filled in with DNA polymerase to create pEX3. The three pEX expression vectors, pEX1, pEX2, pEX3 differ only in their reading frames.

### Unique cloning sites

*Pst* I, *Sal* I, *Bam* HI, *Sma* I, *Eco* RI (pEX2 only)

### Vector Size

5.8 Kb

| Product | Size | Cat. # |
|---|---|---|
| pEX 1 | 25 µg | 6150-1 |
| pEX 2 | 25 µg | 6151-1 |
| pEX 3 | 25 µg | 6152-1 |

### References


1. Stanley, K. K. & Luzio, J. P. (1984) *EMBO J.* **3**:1429.
2. Zabeau, M. & Stanley, K. K. (1982) *EMBO J.* **1**:1217.
3. Sugimoto, K., *et al.* (1977) *J. Mol. Biol.* **111**:487.

# pKK233-2

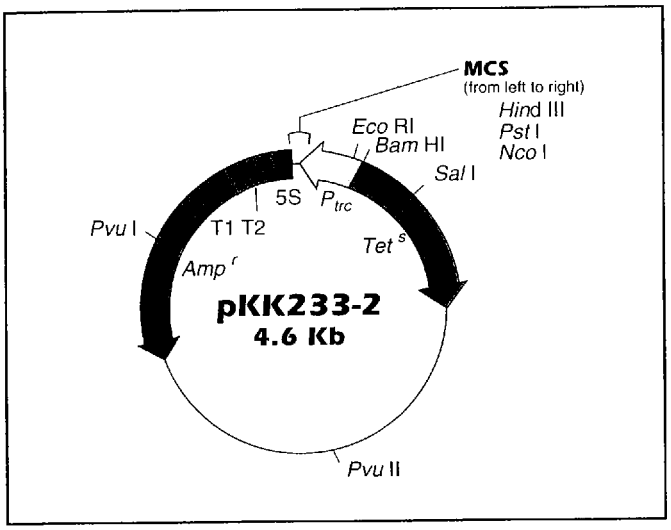



**Figure 12.4** Expression vector pKK233-2

# pKK338-1

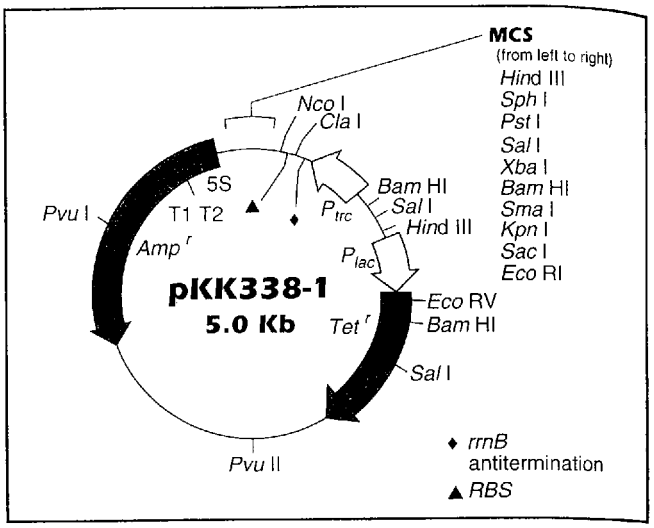



**Figure 12.5** Expression vector pKK338-1

**MCS**

-35 -10
5'...TTGACAATTAATCATCCGGCTCGTATAATGTGTGGAATTGTGAGCGGA

RBS Pst I
TAACAATTTCACACAGGAAACAGACCATGGCTGCAGCCAAGCTTG
Nco I Hind III

rrnB
GCTGTTTTGGC...3'

| Product | Size | Cat. # |
|---|---|---|
| pKK233-2 | 25 µg | 6003-1 |

| Product | Size | Cat. # |
|---|---|---|
| pKK338-1 | 25 µg | 6155-1 |

## Description

pKK233-2 is designed for high-level expression of cloned genes and cDNAs in *E. coli* strains which are *lac* I$^q$ (e.g., JM105). pKK233-2 features the strong IPTG-inducible $P_{trc}$ (trp/lac fusion) promoter (17 base pair spacing between the trp-35 region and the lac UV5 -10 region), the *lacZ* ribosome binding site, and an ATG initiation codon accessible by cleavage at the unique *Nco* I cloning site. The *E. coli rrn*B (5S rRNA) T1 and T2 transcription terminators are downstream of the Multiple Cloning Site to prevent read-through and overexpression of the *amp* gene.

## Unique cloning sites

*Nco* I, *Pst* I, *Hind* III

## Vector Size

4.6 Kb

## References


1. Amann, E. & Brosius, J. (1985) *Gene* **40**:183.
2. Straus, D. & Gilbert, W. (1985) *Proc. Natl. Acad. Sci. USA* **82**:2014
3. Shimizu, Y., *et al.* (1988) *Gene* **65**:141.
4. Kozak, M. (1983) *Microbiol. Rev.* **47**:1.


## Description

pKK338-1 is an "ATG" vector containing a strong trc promoter, and a slightly weaker *lac*UV5* promoter for high-level expression of cloned proteins. pKK338-1 contains *rrn*B anti-termination sequences downstream of the trc promoter, a synthetic Ribosome Binding Site (RBS) followed by a unique *Nco* I site, the pUC18 polylinker region followed by the 5S rRNA gene, and T1 and T2 transcription terminators from *rrn*B.

* The *lac*UV5 promoter does not contain the operator sequence and is not intended for expression.

## Unique cloning sites

*Nco* I, *Eco* RI, *Sac* I, *Kpn* I, *Sma* I, *Xba* I, *Pst* I, *Sph* I

## Vector Size

5.0 Kb

## Reference


1. Vector: A survey of Molecular Cloning Vectors and their uses, edited by Rodriguez, R.L., and Denhardt, D.T., pp. 213-221 (1988).

# pKC30

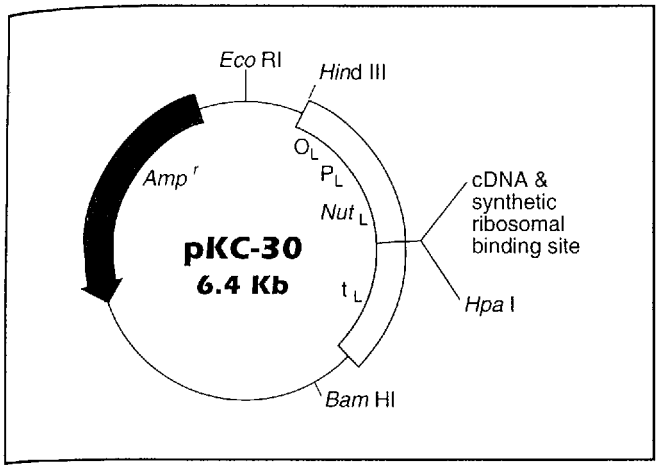



**Figure 12.6** Expression vector pKC30

| Product | Size | Cat. # |
|---|---|---|
| pKC30 | 25 µg | 6103-1 |

## Description

pKC30 is a thermoinducible expression vector designed for over-expresssion of eukaryotic cDNA containing its own translational regulatory information (e.g. RBS and ATG). pKC30 is a pBR322 derivative with a 2.4 Kb *Hind* III-*Bam* HI restriction fragment from λ phage containing the operator ($O_L$), promoter ($P_L$) and N recognition site (Nut L). A unique *Hpa* I restriction site is located within the N gene coding region resulting in an N fusion protein upon cloning at this site. The cDNA and ribosome binding site are located within the *Hpa* I site of the N gene region. The $P_L$ promoter is repressed at 30°C and thermoinduced at 42°C.

**Unique cloning site**
*Hpa* I

**Vector Size** 6442 bp

## Reference

1. Ho, Y. S., *et al.* (1982) *J. Biol. Chem.* **257**:9128.

# pKT279, pKT280, pKT287

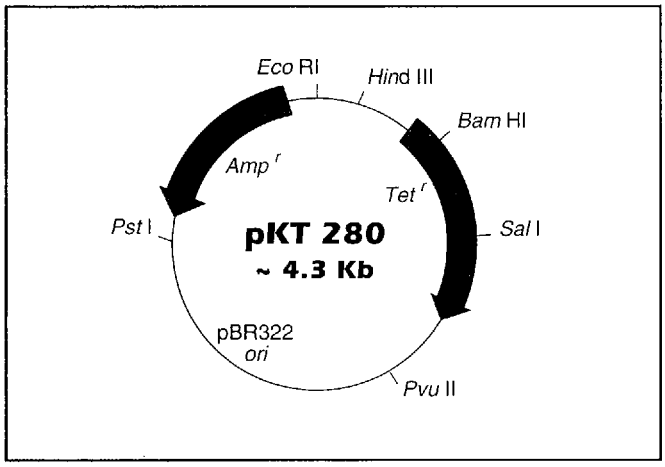



**Figure 12.7** Expression/secretion vector pKT280

| Product | Size | Cat. # |
|---|---|---|
| pKT279 | 25 µg | 6121-1 |
| pKT280 | 25 µg | 6106-1 |
| pKT287 | 25 µg | 6122-1 |

## Description

The three pKT vectors, pKT279, pKT280, and pKT287 are designed for the secretion of cloned gene products into the *E. coli* periplasm. The unique *Pst* I cloning site allows fusion of insert DNA to the promoter and signal peptide sequence of the *E. coli* β-lactamase gene. Tetracycline resistance is available for the amplification and selection of clones in *E. coli*. The three pKT secretion vectors pKT279, pKT280, and pKT287 differ only in their reading frames.

**Unique cloning site**
*Pst* I

**Vector Size** 4.3 Kb

## Reference

1. Talmadge, K., Stahl, S. & Gilbert, W. (1980) *Proc. Natl. Acad. Sci. USA* **77**:3369.

**pKT 279, 280, 287 Secretion Vectors**

**pKT 279**
ATG AGT ATT CAA CAT TTC CGT GTC GCC CTT ATT CCC TTT TTT
GCG GCA TTT TGC CTT CCT GTT TTT GCT CAC CG**C TGC AG**
*Pst* I

**pKT 280**
ATG AGT ATT CAA CAT TTC CGT GTC GCC CTT ATT CCC TTT TTT
GCG GCA TTT TGC CTT CCT GTT TTT GCT **CAC CCG CTG CAG**
*Pst* I

**pKT 287**
ATG AGT ATT CAA CAT TTC CGT GTC GCC CTT ATT CCC TTT TTT
GCG GCA TTT TGC CTT CCT GTT TTT GCT **CAC CCA GAA ACG**
**GCT GCA G**
*Pst* I

* Sequence following signal peptide sequence is in bold.

# pT3/T7-1

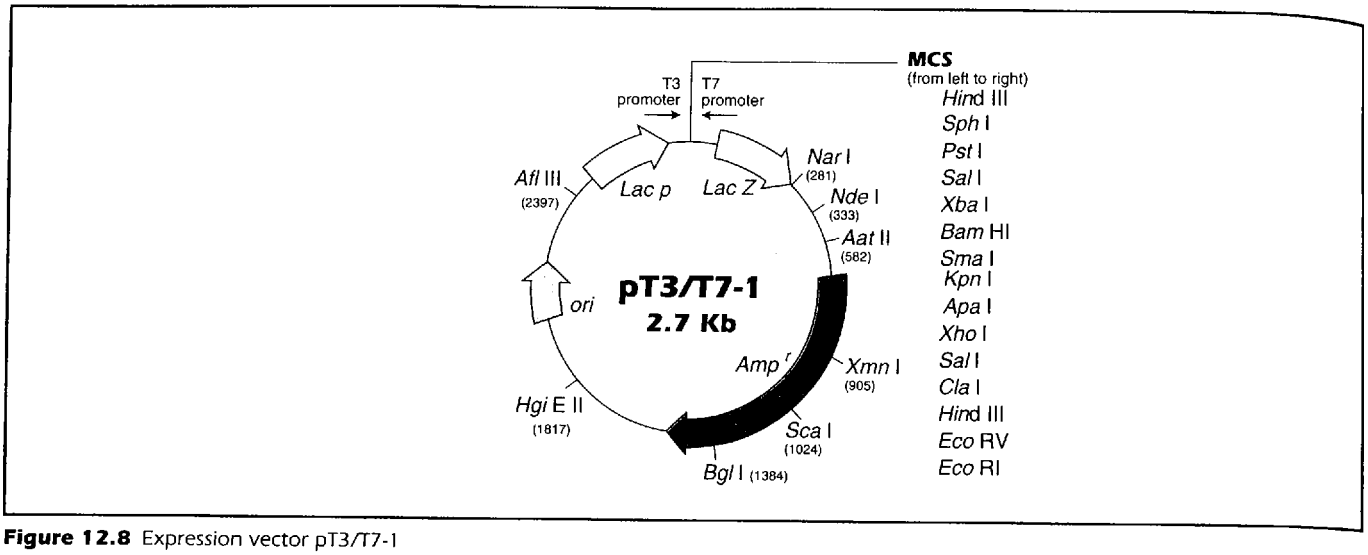
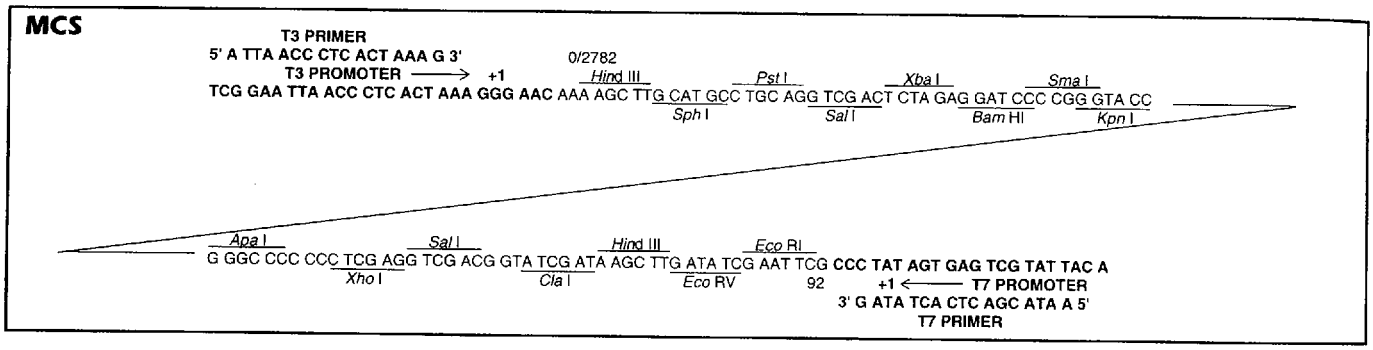



**Figure 12.8** Expression vector pT3/T7-1




12

## Description

pT3/T7-1 is a prokaryotic expression vectors containing diametrically opposed T3 and T7 promoters on either side of the the Multiple Cloning Site. This allows for convenient generation of strand-specific probes by *in vitro* transcription. Recombinant plasmids containing cloned genes in the MCS will appear as white colonies, while wild-type colonies will appear as blue colonies, if grown in a suitable bacterial host strain on medium containing X-gal, a chromogenic compound, and IPTG. Antibody screening may be used for detection of β-galactosidase fusion proteins. DNA sequencing of cloned DNA fragments may be accomplished by directional deletion of inserts. pT3/T7-1 is derived from pUC19 and is identical to pT3/T7-2 except in the composition of the Multiple Cloning Sites.

## Multiple cloning sites

*Apa* I, *Bam* HI, *Cla* I, *Eco* RI, *Eco* RV, *Hind* III, *Kpn* I, *Pst* I, *Sal* I, *Sma* I, *Sph* I, *Xba* I, *Xho* I

## Vector Size

2.7 Kb

| Product | Size | Cat. # |
|---|---|---|
| pT3/T7-1 | 25 µg | 6112-1 |

## References


1. Yanisch-Perron, *et al.* (1985) *Gene* **33**:109.
2. Melton, D.A., *et al.* (1984) *Nucleic Acids Res.* **12**:7035.

# pT3/T7-2

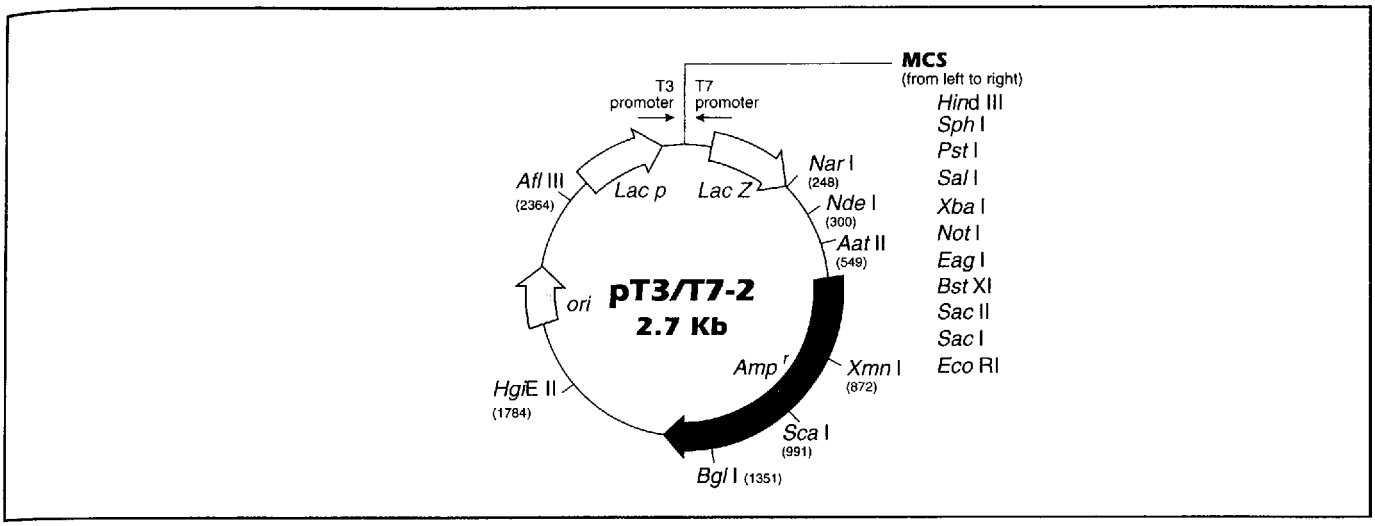
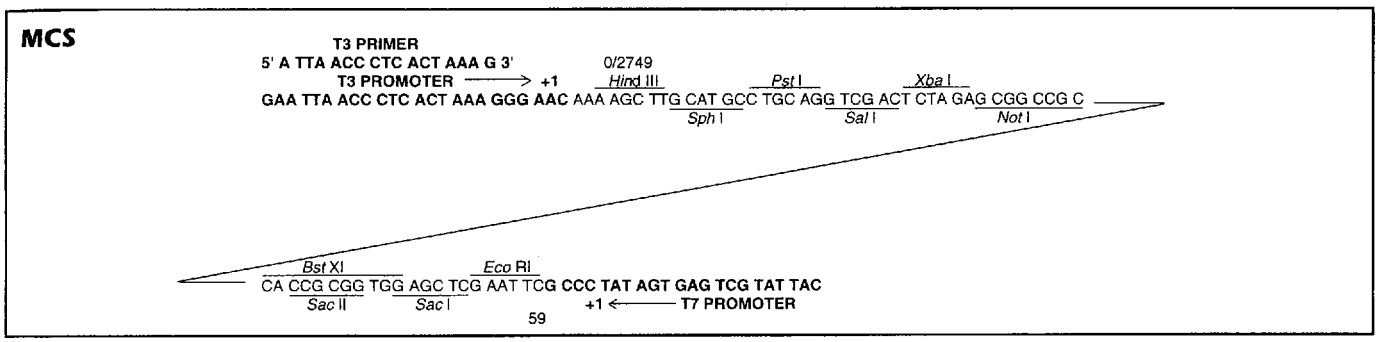



**Figure 12. 9** Expression vector pT3/T7-2




## Description

pT3/T7-2 is a prokaryotic expression vectors containing diametrically opposed T3 and T7 promoters on either side of the the Multiple Cloning Site. This allows for convenient generation of strand-specific probes by *in vitro* transcription. Recombinant plasmids containing cloned genes in the MCS will appear as white colonies, while wild-type colonies will appear as blue colonies, if grown in a suitable bacterial host strain on medium containing X-gal, a chromogenic compound, and IPTG. Antibody screening may be used for detection of β-galactosidase fusion proteins. DNA sequencing of cloned DNA fragments may be accomplished by directional deletion of inserts. pT3/T7-2 is derived from pUC19 and is identical to pT3/T7-1 except in the composition of the Multiple Cloning Sites.

## Multiple cloning sites

*Bst* XI, *Eag* I, *Eco* RI, *Hind* III, *Not* I, *Pst* I, *Sac* I, *Sac* II, *Sal* I, *Sph* I, *Xba* I

## Vector Size

2.7 Kb

| Product | Size | Cat. # |
|---|---|---|
| pT3/T7-2 | 25 µg | 6113-1 |

## References

1. Yanisch-Perron, *et al.* (1985) *Gene* **33**:109.
2. Melton, D.A., *et al.* (1984) *Nucleic Acids Res.* **12**:7035.

# pT3/T7-3

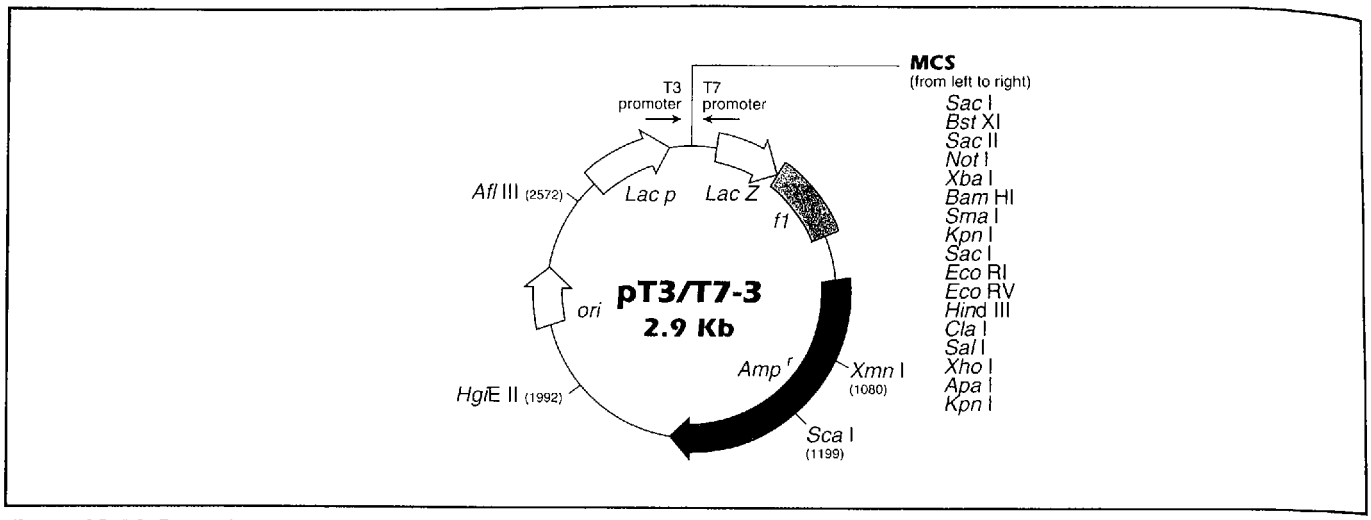
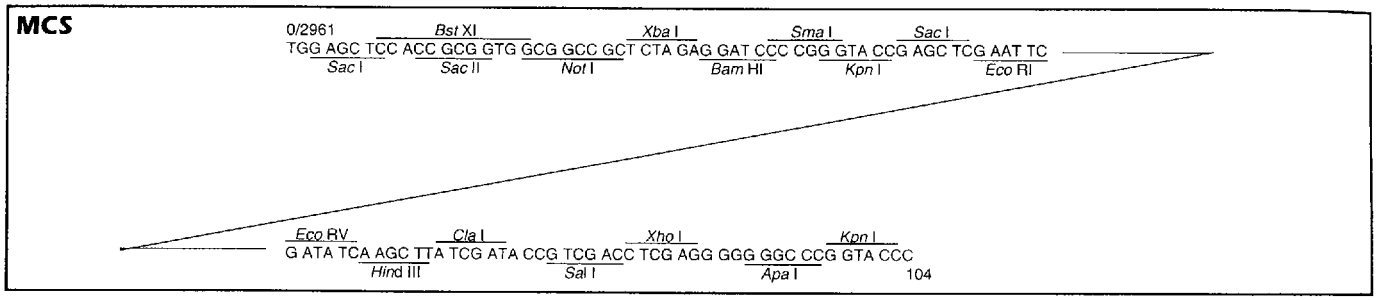



**Figure 12.10** Expression vector pT3/T7-3






## Description

pT3/T7-3 is a prokaryotic expression vector carrying the f1 intergenic region from M13, allowing rescue of single-stranded clones with aid of a helper phage. pT3/T7-3 contains diametrically opposed T3 and T7 promoters on either side of the the Multiple Cloning Site for convenient generation of strand-specific probes by *in vitro* transcription. Recombinant plasmids containing cloned genes in the MCS will appear as white colonies while wild-type colonies will appear blue if grown in a suitable bacterial host strain on medium containing X-gal and IPTG.

Antibody screening may be used for detection of β-galactosidase fusion proteins. DNA sequencing of cloned DNA fragments may be accomplished by directional deletion of inserts. pT3/T7 phagemid is a pUC19 derivative.

## Multiple cloning sites

*Sac* I, *Sac* II, *Bst* XI, *Not* I, *Xba* I, *Bam* HI, *Sma* I, *Kpn* I, *Eco* RI, *Eco* RV, *Hind* III, *Cla* I, *Sal* I, *Xho* I, *Apa* I

**Vector Size** 2.9 Kb

| Product | Size | Cat. # |
|---|---|---|
| pT3/T7-3 | 25 µg | 6114-1 |

# pT3/T7-LUC

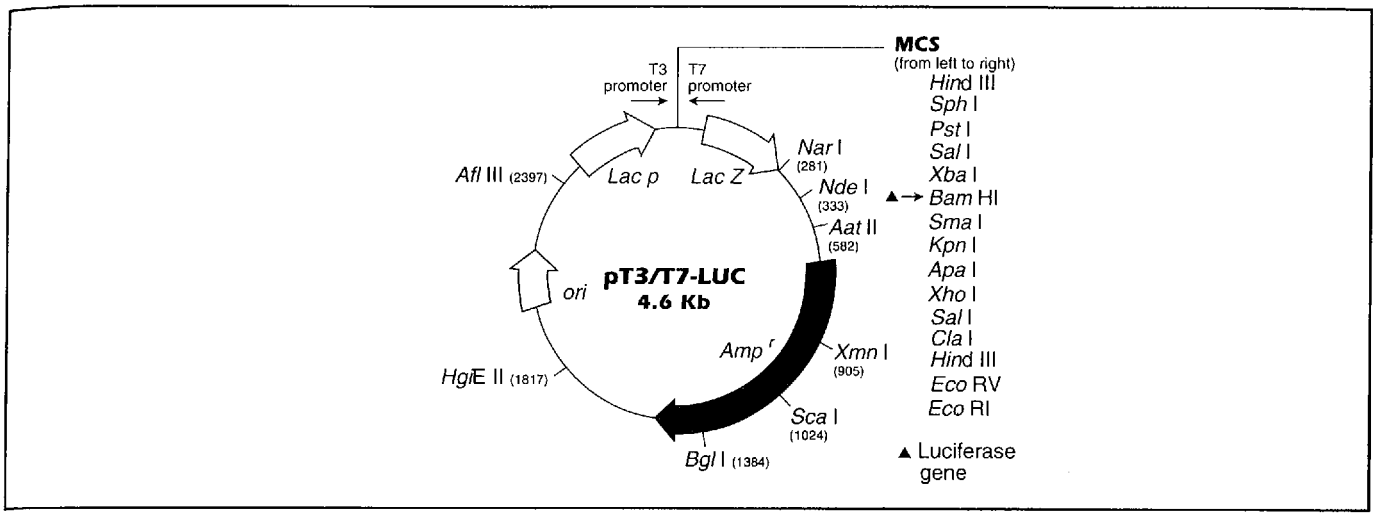
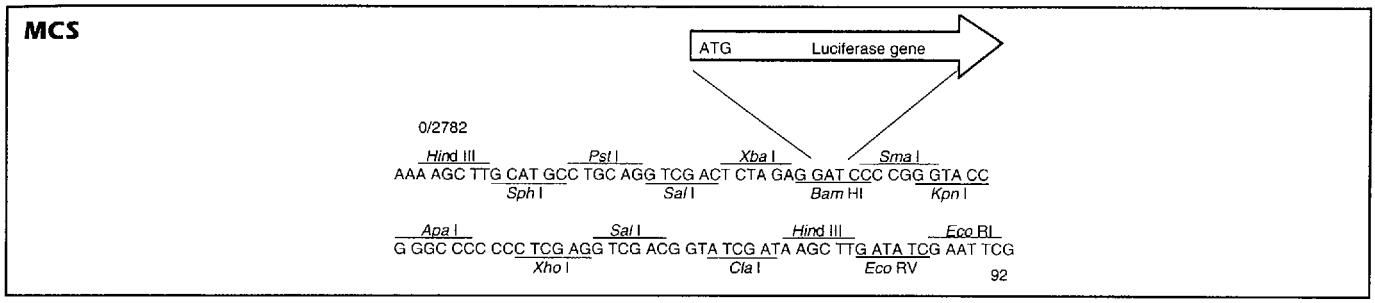



**Figure 12.11** Expression vector pT3/T7-LUC




## Description

pT3/T7-LUC is a prokaryotic expression vector derived from pT3/T7-1 and containing a luciferase reporter gene useful for testing promoter sequences upstream of the *Bam* HI site. pT3/T7-LUC offers one of the most sensitive reporter genes controlled by the *lacZ* promoter sequences. The 1.9 Kb luciferase coding region comes from the firefly luciferase gene. The *luc* gene may be excised by *Bam* HI, *Hind* III or *Sal* I digestion. In addition the T3 and T7 promoters may be used to generate single-stranded RNA probes by *in vitro* transcription.

Luciferase is an enzyme that catalyzes the light-producing chemical reaction of certain bioluminescent organisms. In the presence of luciferin and ATP, the enzyme-bound luciferyl-adenylate complex is formed, which is followed by oxidative decarboxylation. The reaction products are $CO_2$, oxyluciferin, AMP, and light. The light emission may be measured spectrophotometrically or observed on an x-ray film. The luciferase bioassay has been shown to be many times more sensitive than the β-galactosidase or other reporter gene assays. This makes the luciferase gene an important tool for expression studies in micro-organisms, plants, and animals.

| Product | Size | Cat. # |
|---|---|---|
| pT3/T7-LUC | 25 µg | 6170-1 |

**Unique sites within the MCS region**
*Apa* I, *Kpn* I, *Pst* I, *Sma* I, *Xba* I, *Xho* I

**Vector Size** 4.6 Kb

## References


1. DeWet, J. R., *et al.* (1987) *Mol. Cell. Biol.* **7**:725.
2. Ow, D. W., *et al.* (1986) *Science* **234**:856.
3. Rodriguez, J.F., *et al.* (1988) *Proc. Natl. Acad. Sci. USA* **85**:1667.

## EUKARYOTIC EXPRESSION VECTORS

# pEUK-C1

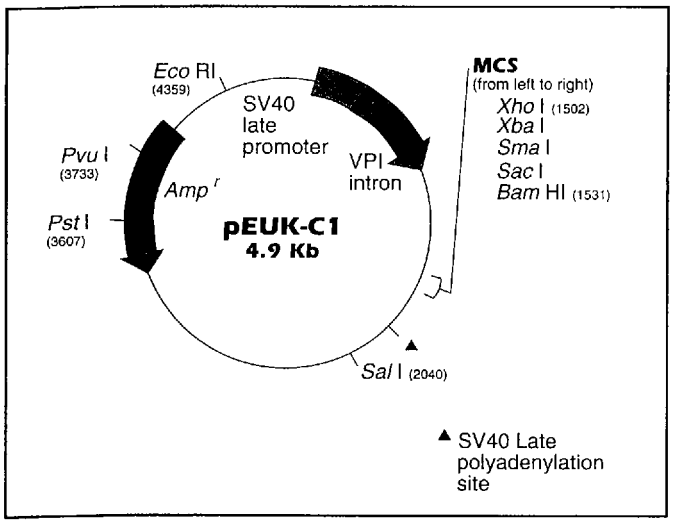



**Figure 12.12** Expression vector pEUK-C1

**MCS**

1502 1531

| Xho I | Xba I | Sma I | Sac I | Bam HI |
|---|---|---|---|---|
| CTC GAG | TCT AGA | CCC GGG | GAG CTC | GGA TCC |

| Product | Size | Cat. # |
|---|---|---|
| pEUK-C1 | 25 µg | 6107-1 |

### Description

pEUK-C1 is a mammalian expression vector designed for high-level expression of eukaryotic genes in COS cells due to the absence of pBR322 "Poison sequences" thus allowing a higher copy number. The expression of cloned genes is under the control of the SV40 late promoter. pEUK-C1 contains SV40 termination, splicing, and polyadenylation elements as well as SV40 and pBR322 origins of replication.

### Unique cloning sites within the MCS region

*Xho* I, *Xba* I, *Sma* I, *Sac* I, *Bam* HI

### Vector Size

4.9 Kb

### References

1. Templeton, D. & Eckhart, N. (1984) *Mol. Cell. Biol.* **4**:817.
2. Lusky, M. & Botchan, M. (1981) *Nature* **293**:79.

# pEUK-C2

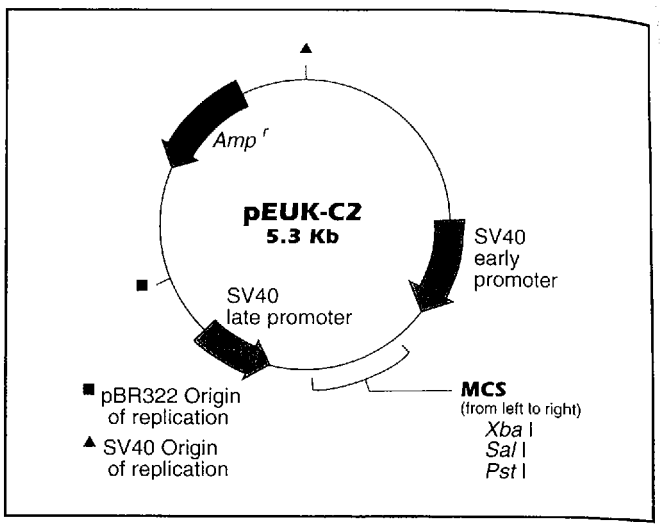



**Figure 12.13** Expression vector pEUK-C2

| Product | Size | Cat. # |
|---|---|---|
| pEUK-C2 | 10 µg | 6001-1 |

### Description

pEUK-C2 is a high efficiency eukaryotic cDNA expression vector. It is distinguished by two diametrically opposed SV40 (Early and Late) promoters. Both strands of the inserted DNA can be transcribed and translated, thus any cDNA inserted into this vector has twice the probability of being expressed. The unique *Pst* I site may be used to construct cDNA libraries. pEUK-C2 contains the SV40 and pBR322 origins of replication. High level expression of eukaryotic genes in COS cells is due to the absence of pBR322 "Poison sequences." The *E. coli* gene for ampicillin resistance makes this vector easy to amplify in *E. coli*.

### Unique cloning sites within the MCS region

*Pst* I, *Sal* I, *Xba* I

### Vector Size

5.3 Kb

### Reference

1. Davis, E., Harpaz, N., Johnson, E. (1990) *JBC* **265** (36):22153.

YEAST CLONING & EXPRESSION VECTORS

# Lambda Max1 & pYEUra3

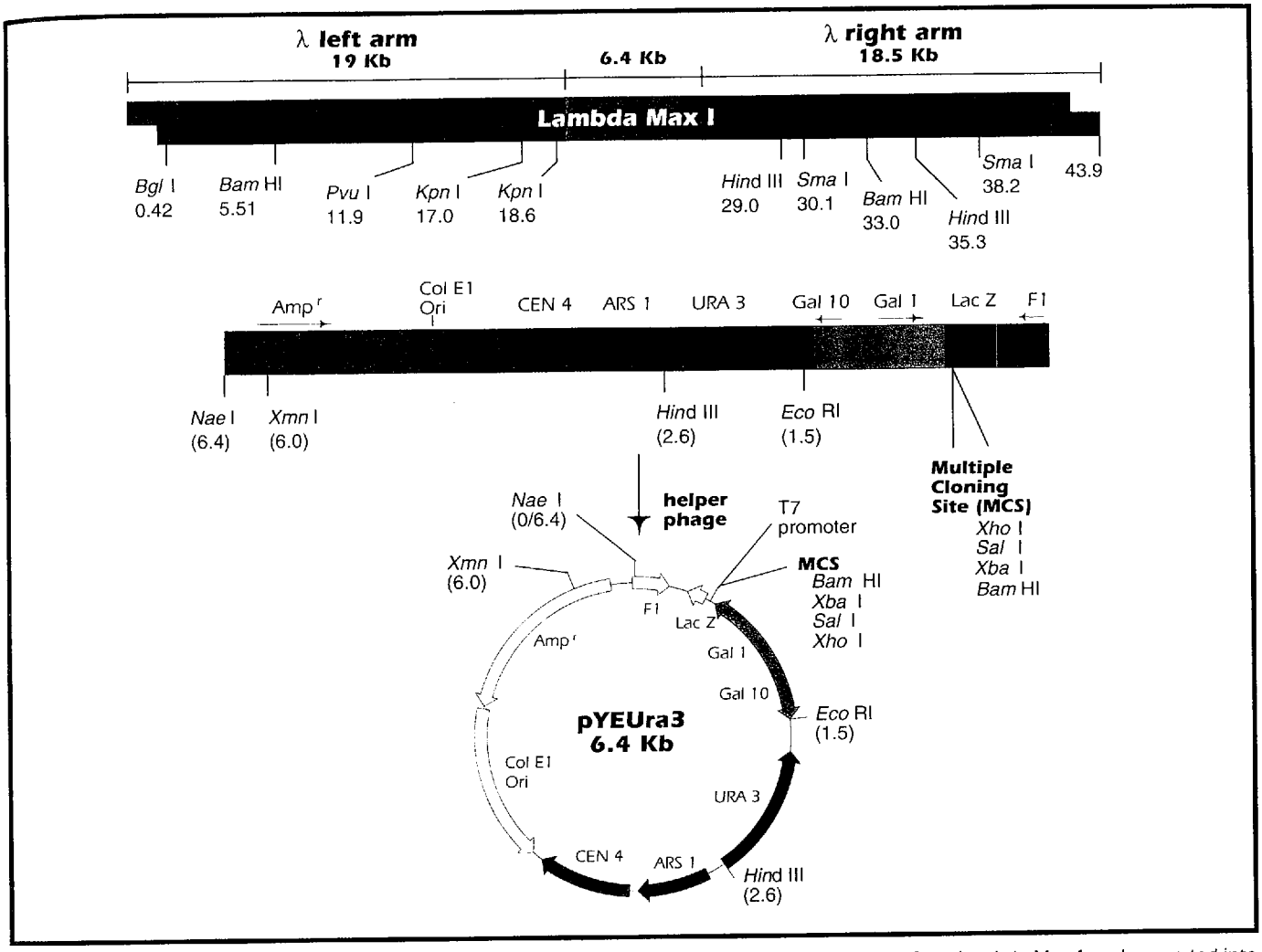



**Figure 12.14** Schematic diagram of Lambda Max 1 and pYEUra3. pYEUra3 sequences can be excised from Lambda Max 1 and converted into the plasmid by helper phage.

Recent molecular analysis of genes reveals a striking similarity in both structure and function between mammalian and yeast genes. Although molecular cloning techniques have elucidated gene structures at an unprecedented rapid pace, the functions of many of these genes remain elusive. Studies are now being conducted to determine gene function in yeast systems, and what is learned from these studies in yeast may eventually be applied to mammalian systems. A number of yeast vectors have been designed and constructed for cloning cDNAs so that their functions may be identified in yeast cells. However, the cloning efficiency in plasmid-type yeast vectors is generally low and this factor seriously limits their utility for these studies. Therefore, we have developed Lambda Max 1 to enable both efficient cloning and yeast expression.

The design of Lambda Max 1 incorporates the properties of lambda phage that make it highly efficient in cloning cDNA, as λgt10 and λgt11 can be, together with several yeast elements that enable propagation and expression of the cloned cDNA in yeast cells.

The yeast elements include the inducible GAL 1 and GAL 10 promoters as well as URA 3, CEN 4, and ARS 1. These yeast elements, together with the multiple cloning site, can be excised and converted into the plasmid pYEUra3 by helper phage for transformation and expression of the cloned cDNA in yeast.

# Lambda Max 1™

- **cDNA cloning vector for yeast expression studies**

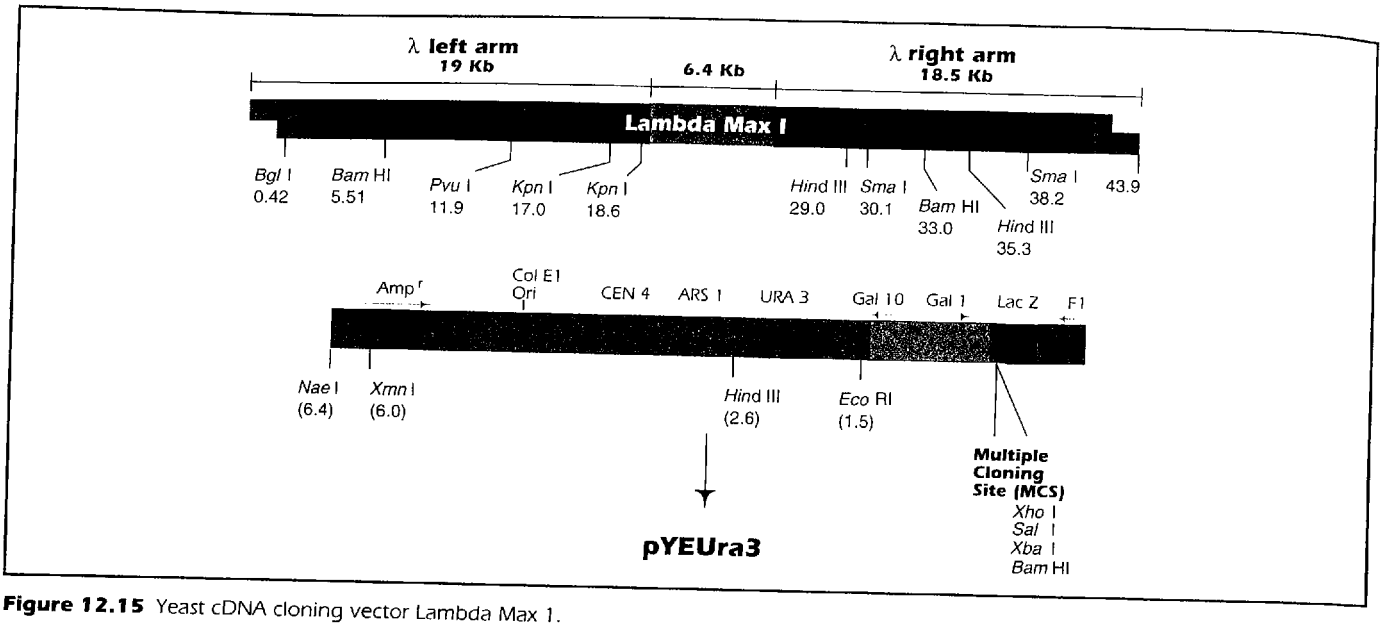



**Figure 12.15** Yeast cDNA cloning vector Lambda Max 1.

12

| Product | Size | Cat. # |
|---|---|---|
| Lambda Max 1 | 20 µg | 6192-1 |
| Lambda Max 1 arms (*Xba* I/*Xho* I) | 10 µg | 6193-1 |
| Lambda Max 1 arms (*Eco* RI-digested) | 10 µg | 6194-1 |

## Description

A unique Yeast cloning vector designed for high-efficiency cloning of cDNA and restriction enzyme digests. Plasmid with the cDNA sequence can be excised with helper phage and converted into the pYEUra3 plasmid by helper phage, thus circumventing subcloning steps. Plasmid cDNA is then ready for mapping and sequencing. Lambda Max1 allows generation of cDNA libraries of high complexity (>106 clones), and clones cDNA up to 7.0 Kb in length.

Lambda Max1 enables directional cloning of cDNA to enhance yeast expression and is ideal for expression studies of mammalian and other eukaryotic genes in yeast, to identify human analogs of yeast genes, and to find a yeast gene equivalent to your mammalian gene. The Lambda Max1 arms are also available separately, digested with either *Xba* I/*Xho* I or with *Eco* RI. Complete protocols and helper phage are included with each product.

## Unique cloning sites

*Xba* I, *Xho* I, *Bam* HI, *Sal* I

## Vector size

44 Kb

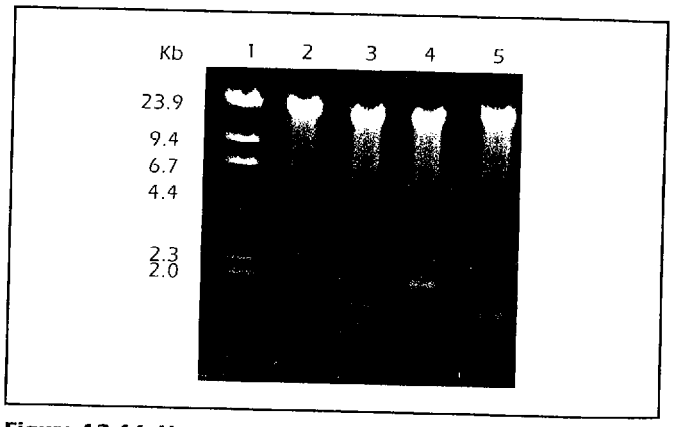



**Figure 12.16 Human HeLa cDNA library in Lambda Max 1.** Four clones (Lanes 2-5) were randomly picked and their inserts excised by *Xba* I and *Xho* I digestion. Lane 1: Lambda DNA/*Hind* III size marker.

# pYEUra3™

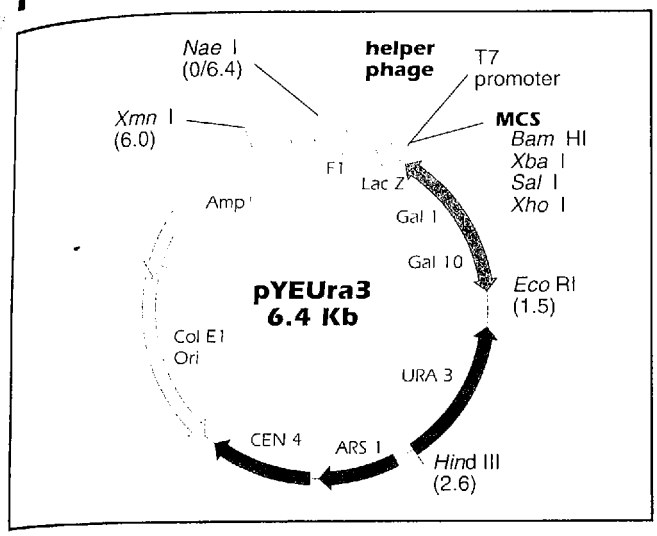



**Figure 12.17** Yeast expression vector pYEUra3.

| Product | Size | Cat. # |
|---|---|---|
| pYEUra3 | 20 µg | 6195-1 |

**Description**

A yeast expression vector with the URA3 marker. pYEUra3 contains the strong Gal 1 and Gal 10 promoters and other elements that can propagate and express cDNA in yeast cells. Also contains selection markers for propagation of plasmids in *E.coli*. Routinely yields frequencies of $10^5$ yeast transformants/µg. A complete protocol is included.

**Unique cloning sites**

*Bam* HI, *Xba* I, *Sal* I, *Xho* I

**Vector size** 6.4 Kb

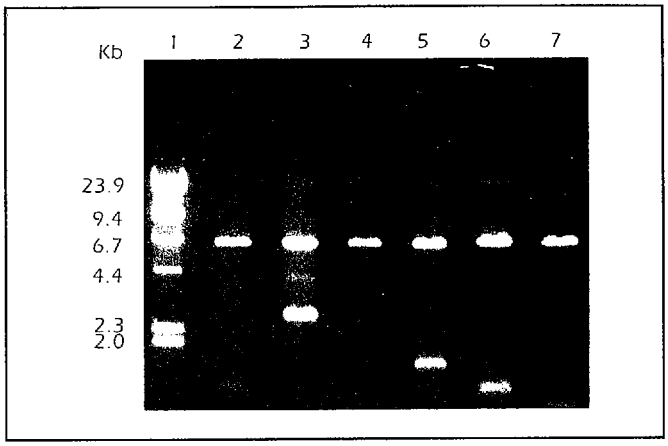



**Figure 12.18 Human HeLa cDNA Library in pYEUra3.** Six colonies (Lanes 2-7) were randomly picked and their inserts excised by *Xba*I/*Xho*I. The Human HeLa cDNA Library was generated by converting the library (Lambda Max 1) into the plasmid pYEUra3 using a helper phage and then transforming the plasmids into bacteria. Lane 1: Lambda DNA/*Hin*d III size marker.

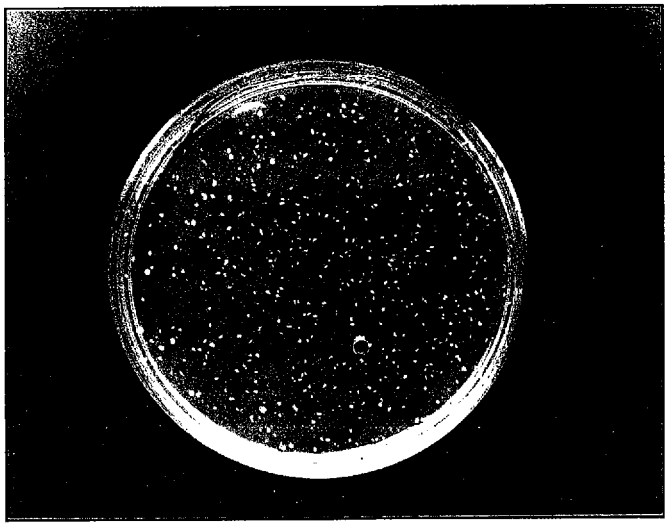


**Figure 12.19 Yeast transformants.** The entire human HeLa cell library in pYEUra3 transformed into competent yeast cells. Approximately $10^5$ transformants were scored per µg of recombinant DNA used.

# pG1

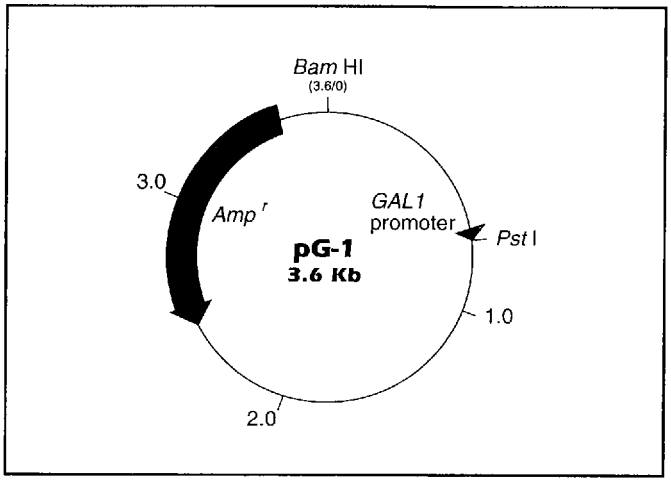



**Figure 12.20** Yeast cDNA cloning vector pG1

| Product | Size | Cat. # |
|---|---|---|
| pG1 | 25 µg | 6135-1 |

## Description

pG1 is a yeast cDNA cloning vector used as the Okayama-Berg "adaptor" for the construction of cDNA expession libaries in *Saccharomyces cerevisiae*. The galactose-inducible GAL1 promoter is on a 0.8 Kb *Bam* HI-*Pst* I fragment upstream of the *Pst* I cloning site. pG1 is used in tandem with pTRP56 in *S. cerevisiae* strain SHY2 for high efficiency cloning and expression of full-length cDNAs in yeast. The ampicillin resistance gene is present for selection in *E. coli*.

## Unique cloning site

*Pst* I

## Vector Size

3.6 Kb

## References

1. Muyajma, A., *et al.* (1984) *Nucleic Acids Res.* **12**:6397.
2. Goff, C. G., *et al.* (1984) *Gene* **27**:35.

# pTRP56

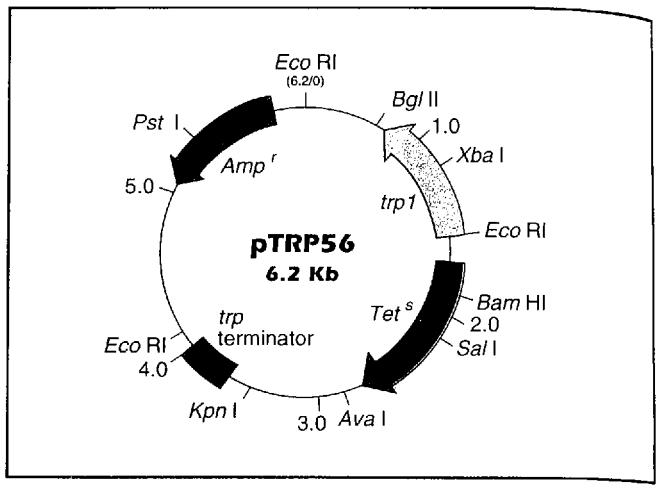



**Figure 12.21** Yeast expression vector pTRP56

| Product | Size | Cat. # |
|---|---|---|
| pTRP56 | 25 µg | 6130-1 |

## Description

pTRP56 is a cDNA yeast cloning vector designed to be used in conjunction with pG1 as the Okayama-Berg "primer" for the construction of cDNA expression libraries in *Saccharomyces cerevisiae*. pTRP56 carries the ARS yeast element allowing autonomous replication in *S. cerevisiae*. The TRP1 selection marker is used for selection in *S. cerevisiae* strain SHY2. Transformants are selected by complementation of Trp1⁻ strains on media which lack tryptophan. The resulting cDNA construct can be propagated in either *Saccharomyces* or *E. coli*. Ampicillin resistance is present for selection in *E. coli*.

## Vector Size

6.2 Kb

## References

1. Muyajma, A., *et al.* (1984) *Nucleic Acids Res.* **12**:6397.
2. Goff, C. G., *et al.* (1984) *Gene* **27**:35.

# pYACneo

## Yeast artificial chromosome cloning vector

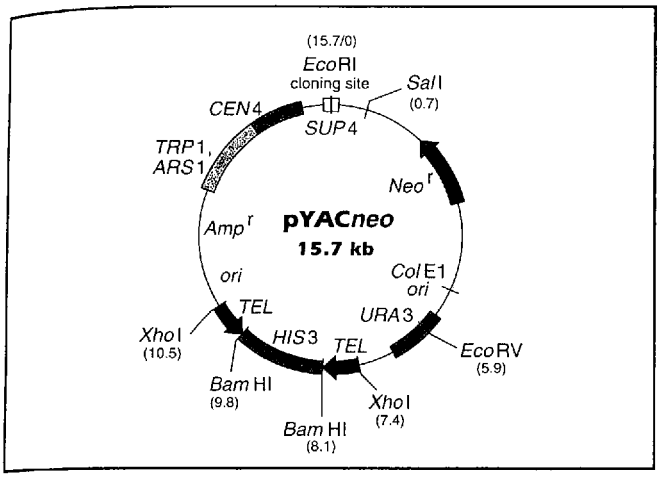



**Figure 12.22**

| Product | Size | Cat.# |
|---|---|---|
| pYACneo | 20 µg<br>120 µg | 6190-1<br>6190-2 |
| pYACneo arms | 20 µg<br>100 µg | 6191-1<br>6191-2 |

### Description

Yeast artificial chromosome (YAC) cloning vector for use in cloning large DNA fragments up to 1.0 Megabase to facilitate genome mapping and sequencing projects.

pYACneo contains *Xho* I sites for plasmid rescue of both ends of the insert, and the neomycin resistance gene to allow transfer of a positive YAC clone to mammalian cells. Upon insertion of *Eco* RI digested fragments, the sup4-O yeast nonsense suppressor becomes inactivated; this reduces the background of non-recombinants and allows for their visual detection as light-pink rather than red.

The pYAC neo arms, prepared by digestion of pYACneo with *Eco* RI and *Bam* HI followed by dephosphorylation, are also available separately. Each product includes *Eco* RI control DNA insert, a complete protocol for vector preparation, and an annotated bibliography for developing cloning strategies.

**Vector size** 15.7 Kb

### References

1. Burke, D.T. *et al.* (1987) *Science* **236**:806.
2. Albertson, *et al.* (1990) *Proc. Natl. Acad. Sci.* **87**:4256.
3. Anand, *et al.* (1990) *Nucleic Acids Res.* **18**:1951.

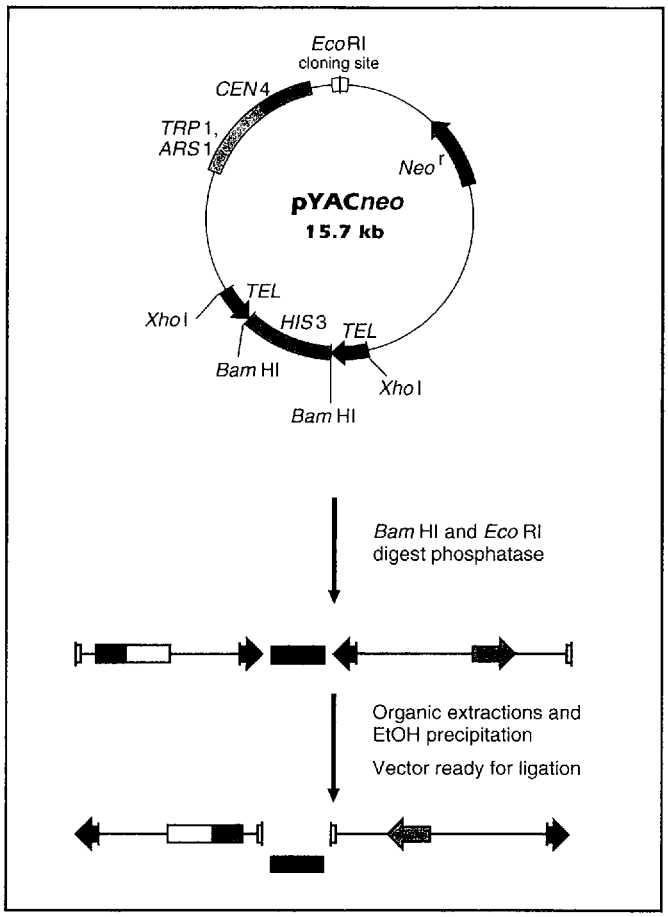



**Figure 12.23**

## MAMMALIAN EXPRESSION VECTORS

# pMAM

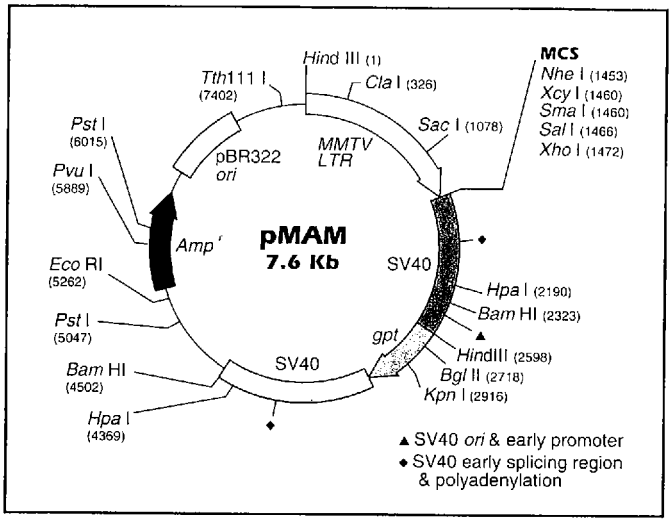



**Figure 12.24** Expression vector pMAM

# pMAM*neo*

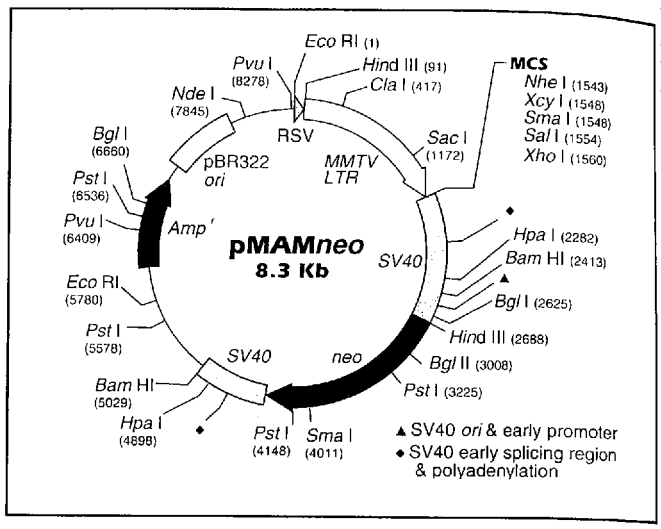



**Figure 12.25** Expression vector pMAM*neo*

12

**MCS**

1453 1460 1470

AAGCTAGCCCGGGGTCGACCTCGAGGG

*Nhe* I *Xcy* I *Sal* I *Xho* I

*Sma* I

| Product | Size | Cat. # |
|---|---|---|
| pMAM | 20 µg | 6100-1 |

**Description**

pMAM is the parental plasmid of the pMAM mammalian expression vector family. pMAM offers dexamethasone-inducible expression driven by the MMTV-LTR promoter and contains the *E. coli* gpt gene, which allows selection of transformants in HAT medium, and the SV40 ori, splicing, and polyadenylation elements.

**Unique cloning sites within the MCS region**

*Nhe* I, *Xcy* I, *Sma* I, *Sal* I, *Xho* I

**Vector Size** 7.6 Kb

**MCS**

1541 1550 1560

AAGCTAGCCCGGGGTCGACCTCGAGGG

*Nhe* I *Xcy* I *Sal* I *Xho* I

| Product | Size | Cat. # |
|---|---|---|
| pMAM*neo* | 25 µg | 6104-1 |

**Description**

pMAM*neo* is a mammalian expression vector designed for high level expression of eukaryotic genes. pMAM*neo* contains the RSV-LTR enhancer linked to the dexamethasone-inducible MMTV-LTR promoter. This construction yields controllable yet high level expression of cloned genes in the presence of dexamethasone. The SV40 splicing and polyadenylation sites provide RNA processing in mammalian cells while the *E. coli neo* gene, driven by the SV40 early promoter allows selection of transformants growing in media containing G418 antibiotics.

**Unique cloning sites within the MCS region**

*Nhe* I, *Sal* I, *Xho* I

**Vector Size** 8.3 Kb

**References**

1. Lee, F., *et al.* (1981) *Nature* **294**:228 .
2. Sardet, C., Franchi, A. & Pouyssegur, J. (1989) *Cell* **56**:271.

# pMAM*neo*-BLUE

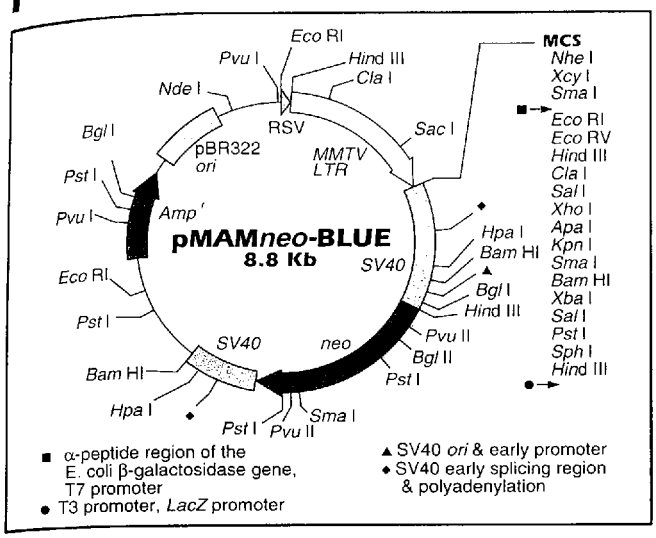



**Figure 12.26** Expression vector pMAM*neo*-BLUE

# pMAM*neo*-CAT

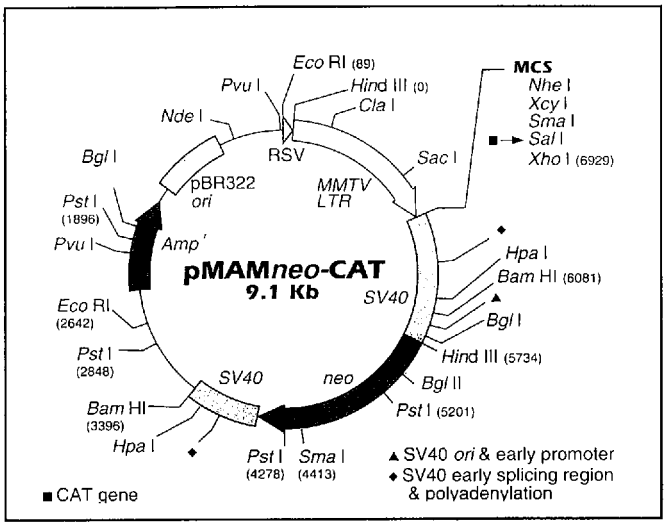
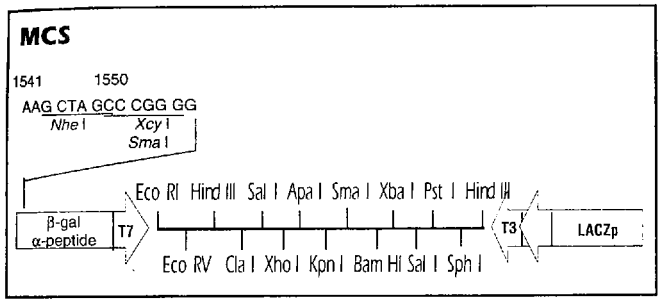
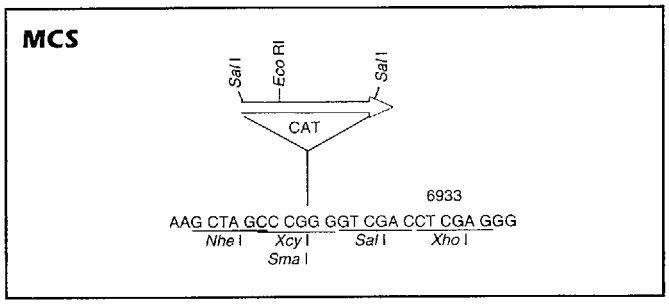



**Figure 12.27** Expression vector pMAM*neo*-CAT




| Product | Size | Cat. # |
|---|---|---|
| pMAM*neo*-BLUE | 25 µg | 6105-1 |

| Product | Size | Cat. # |
|---|---|---|
| pMAM*neo*-CAT | 25 µg | 6141-1 |

## Description

pMAM*neo*-BLUE is a mammalian expression vector derived from pMAM*neo* by the insertion of a 540 bp fragment containing the *lacZ* promoter, the α-peptide regions of the β-galactosidase gene, the T3 and T7 RNA polymerase promoters, and a polylinker region cloned into the multiple cloning site of pMAM*neo*. Recombinant transformants in *E. coli* may be immuno- or blue/white colony screened. Cloned gene expression is regulated by induction with dexamethasone. The T3 and T7 promoters provide *in vitro* transcription of cloned gene fragments.

**Unique cloning sites within the MCS region**
*Nhe* I, *Eco* RV, *Xho* I, *Kpn* I, *Xba* I

**Vector Size** 8.8 Kb

## Description

pMAM*neo*-CAT is a eukaryotic expression vector and by virtue of an easily-detected chloramphenicol acetyltransferase (CAT) activity is useful in three separate ways: (1) as a positive transfection control; (2) for testing the function of cloned regulatory sequences; and (3) as a method of providing fusion polypeptides for the stabilization of cloned gene products. The CAT gene found in pMAM*neo*-CAT is from the *E. coli* Tn9 transposon. The RSV-LTR enhancer is linked to the dexamethasone-inducible MMTV-LTR promoter for controlled yet high-level expression of CAT. pMAM*neo*-CAT is equipped with the SV40 ori, splicing, and polyadenylation sites.

**Unique cloning sites within the MCS region**
*Nhe* I, *Xho* I

**Vector Size** 9.1 Kb

**References**

1. Close, T.J. & Rodriguez, R. (1982) *Gene* **20**:305.
2. Lee, F., *et al.* (1981) *Nature* **294**:228.

# pMAM*neo*-LUC

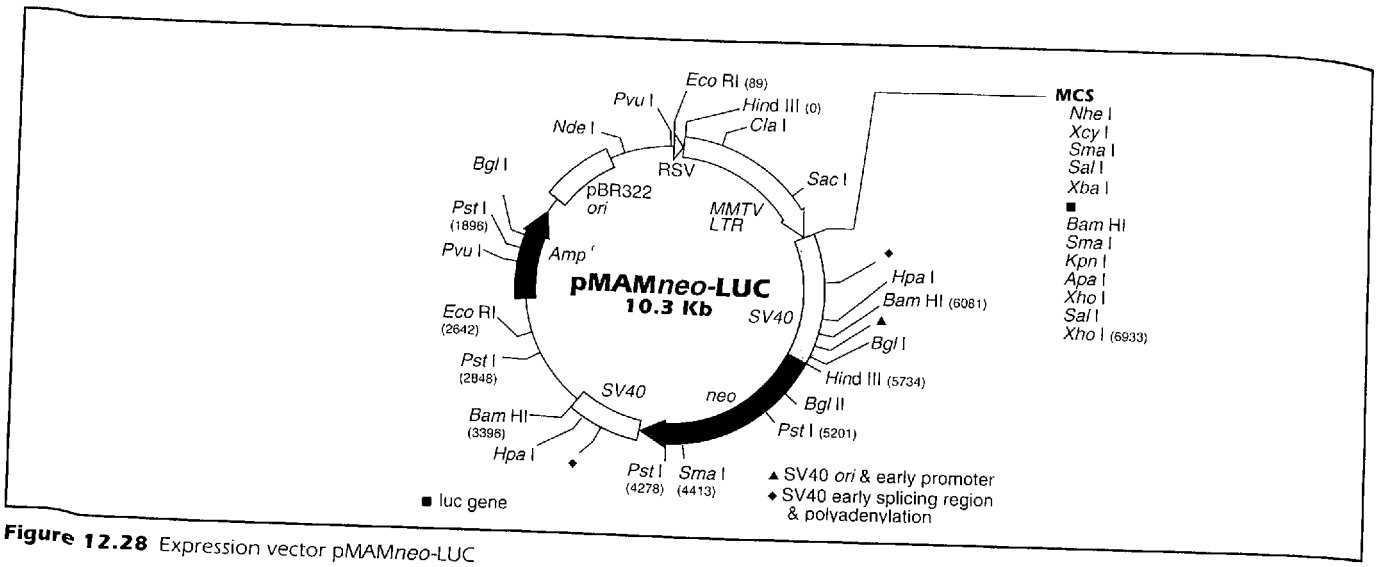
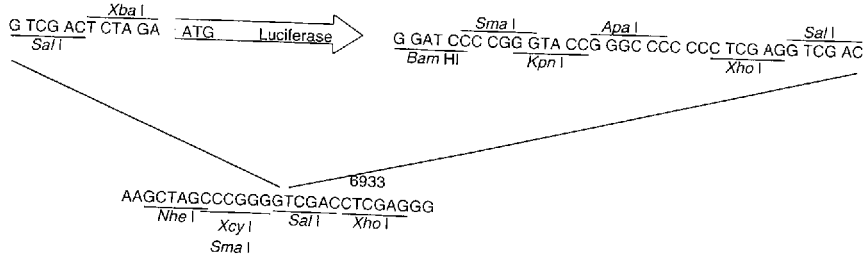



**Figure 12.28** Expression vector pMAM*neo*-LUC

12

**MCS**




## Description

pMAM*neo*-LUC is a mammalian expression vector derived from pMAM*neo* that contains the firefly luciferase reporter gene. pMAM*neo*-LUC offers one of the most sensitive reporter genes available. The 1.9 Kb luciferase coding region of the firefly luciferase gene was cloned into the pMAM*neo* *Sal* I site. pMAM*neo*-LUC contains SV40 ori, splicing, and polyadenylation elements. In addition, pMAM*neo*-LUC features stabilization of cloned gene products by fusion to luciferase. The pBR322 *ori* and *amp* genes allow easy propagation and amplification in *E. coli*.

Luciferase is an enzyme that catalyzes the light-producing chemical reaction of some bioluminescent organisms. In the presence of luciferin and ATP, the enzyme-bound luciferyl-adenylate complex is formed, which is followed by oxidative decarboxylation. The reaction products are $CO_2$, oxyluciferin, AMP, and light. The light emission may be measured spectrophotometrically or observed on an x-ray film. The luciferase bioassay has been shown to be about 1000 times more sensitive than the β-galactosidase or other reporter gene assays. The luciferase gene is therefore a useful tool for expression studies in microorganisms, plants, and animals.

| Product | Size | Cat. # |
|---|---|---|
| pMAM*neo*-LUC | 25 µg | 6171-1 |

**Unique cloning sites within the MCS region**
*Xba* I, *Kpn* I

**Vector Size** 10.3 Kb

## References


1. DeWet, J.R., *et al.* (1987) *Mol. Cell. Biol.* **7**:725.
2. Ow, D.W., *et al.* (1986) *Science* **234**:856.
3. Rodriguez, J.F., *et al.* (1988) *Proc. Natl. Acad. Sci. USA* **85**:1667.

# pCH110

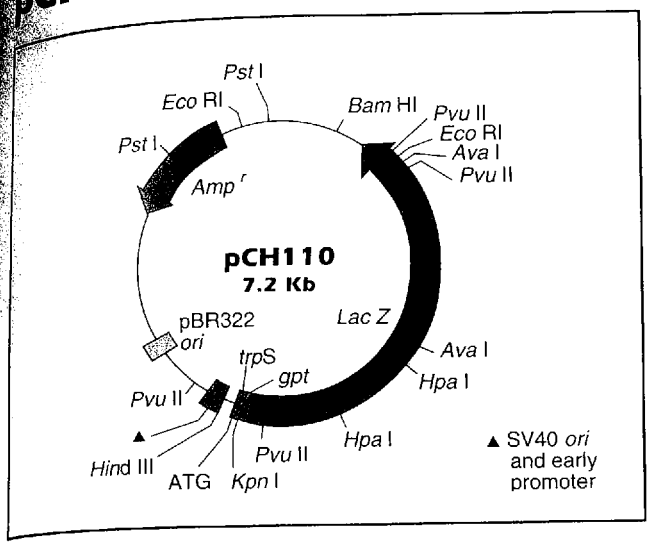



**Figure 12.29** Expression vector pCH110

| Product | Size | Cat. # |
|---|---|---|
| pCH110 | 25 µg | 6101-1 |

## Description

pCH110 is a transient expression vector containing a functional *lacZ* gene expressed from either the SV40 early promoter or from the *E. coli* gpt promoter. pCH110 is especially suited for transient expression of β-galactosidase in COS cells. The β-gal level can be monitored for studying mammalian cell expression or as an indicator of the effects of altered cell culture conditions on gene expression. pCH110 contains both the SV40 and pBR322 origins of replication, the *amp* gene for selection in *E. coli*, and the SV40 early promoter fused to the *E. coli lacZ* gene. pCH110 may be used as a positive control to normalize cloned gene expression in COS cells, as well as for the determination of recombinant transformation efficiency by β-gal activity assays. When pCH110 is co-transfected with a second plasmid, it can serve as an internal marker for monitoring and normalizing transient and stable expression between different experiments in eukaryotic cells. pCH110 allows detection of recombinant expression by an easy colorimetric assay for β-galactosidase via fusion of cloned genes to a segment of the *lacZ* gene.

## Unique cloning sites

*Hind* III, *Kpn* I

## Vector Size

7.3 Kb

## References


1. Hall, C. V., *et al.* (1983) *J. Mol. Appl. Gen.* **2**:101.
2. Miller, J. H. in Experiments in Molecular Genetics (Cold Spring Harbor Laboratory) (1972).
3. Herbomel, P., *et al.* (1984) *Cell* **39**:653.
4. Lee, F. *et al.* (1984) *Nucleic Acids Res.* **12**:4191.


# pNASSβ

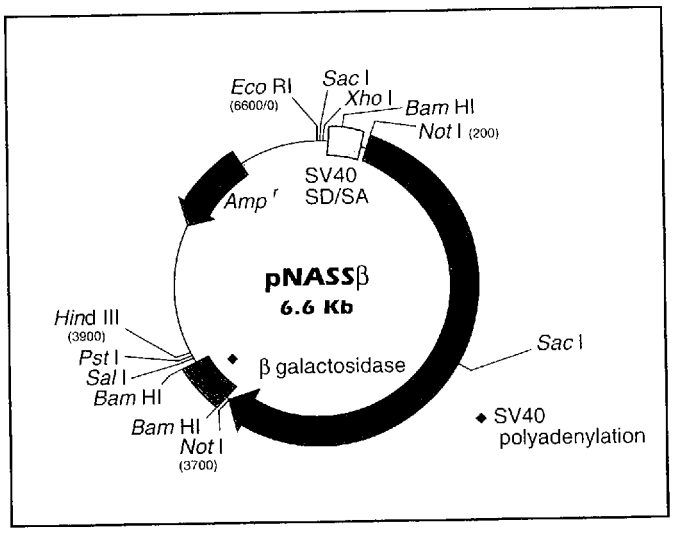



**Figure 12.30** Expression vector pNASSβ

| Product | Size | Cat. # |
|---|---|---|
| pNASSβ | 20 µg | 6175-1 |

## Description

pNASSβ is a promoterless mammalian expression vector that allows cloning and testing of promoters using β-galactosidase expression. pNASSβ contains the SV40 RNA splice site, a polyadenylation signal, and the *E. coli* β-gal gene. The β-gal gene is located within a pair of *Not* I restriction sites for easy excision and replacement with another gene of interest.

## Unique cloning sites

*Eco* RI, *Xho* I, *Sal* I, *Pst* I, *Hind* III

## Vector Size

6.6 Kb

## References


1. MacGreggor & Caskey (1989) *Nucleic Acids Res.* 17:2365.
2. Norton & Coffin (1985) *Mol. and Cell Biol.* **5**:281.
3. Alam, J. (1990) *Anal. Biochem.* **188**:245-254.

# pADβ

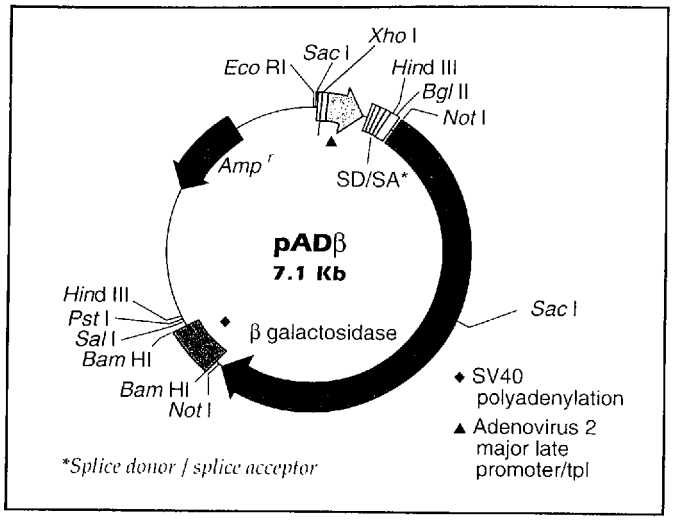



**Figure 12.31** Expression vector pADβ

| Product | Size | Cat. # |
|---|---|---|
| pADβ | 25 µg | 6176-1 |

## Description

pADβ is a mammalian expression vector designed for the expression of β-galactosidase in mammalian cells as transcribed by the Adenovirus promoter. pADβ contains an RNA splice site, a polyadenylation signal, and the *E. coli* β-galactosidase gene. The β-gal gene is located within a pair of *Not* I restriction sites allowing easy excision and replacement with another gene of interest. pADβ may be used for optimizing electroporation conditions, as a reference plasmid for reporter gene constructs, for "enhancer-trap" vectors, or for analyzing *cis* acting elements and trans acting factors in conjunction with an effective β-gal stain.

**Cloning site**
*Not* I
**Unique sites**
*Eco* RI, *Pst* I

**Vector Size** 7.1 Kb

## References

1. MacGreggor & Caskey (1989) *Nucleic Acids Res.* **17**:2365.
2. Norton & Coffin (1985) *Mol. and Cell Biol.* **5**:281.
3. Alam, J. (1990) *Anal. Biochem.* **188**:245-254.

# pCMVβ

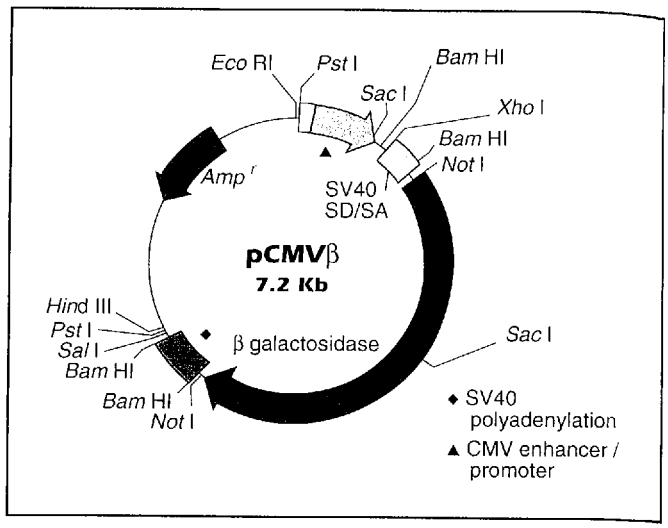



**Figure 12.32** Expression vector pCMVβ

| Product | Size | Cat. # |
|---|---|---|
| pCMVβ | 25 µg | 6177-1 |

## Description

pCMVβ is a mammalian expression vector designed for the expression of β-galactosidase in mammalian cells as transcribed by the Cytomegalovirus promoter. pCMVβ contains an RNA splice site, a polyadenylation signal and the *E. coli* β-gal gene. The β-gal gene is located within a pair of *Not* I restriction sites for excision and replacement with another gene of interest. pCMVβ may be used for optimizing electroporation conditions, as a reference plasmid for reporter gene constructs, for "enhancer-trap" vectors, or for analyzing cis acting elements and trans acting factors in conjunction with an effective β-gal stain.

**Cloning site**
*Not* I

**Vector Size** 7.2 Kb

## References

1. MacGreggor & Caskey (1989) *Nucleic Acids Res.* **17**:2365.
2. Norton & Coffin (1985) *Mol. and Cell Biol.* **5**:281.
3. Alam, J. (1990) *Anal. Biochem.* **188**:245-254.

## pSVβ

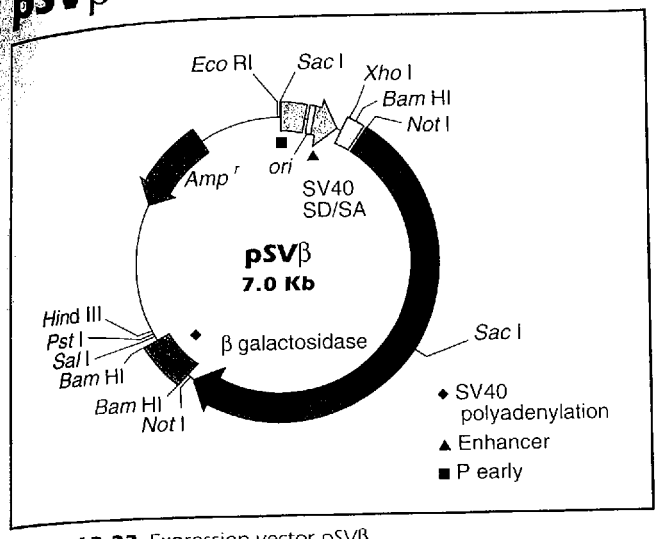



**Figure 12.33** Expression vector pSVβ

| Product | Size | Cat. # |
|---|---|---|
| pSVβ | 25 µg | 6178-1 |

### Description

pSVβ is a mammalian expression vector designed for the expression of β-galactosidase in mammalian cells as transcribed by the SV40 promoter. pSVβ contains an RNA splice site, a polyadenylation signal, and the *E. coli* β-gal gene. The β-gal gene is located within a pair of *Not* I restriction sites used for easy excision and replacement with another gene of interest. pSVβ may be used for optimizing electroporation conditions, as a reference plasmid for reporter gene constructs, for "enhancer-trap" vectors, or for analyzing *cis* acting elements and trans acting factors in conjunction with an effective β-gal stain.

### Cloning site

*Not* I

### Vector Size

7.0 Kb

### References


1. MacGreggor & Caskey (1989) *Nucleic Acids Res.* **17**:2365.
2. Norton & Coffin (1985) *Mol. and Cell Biol.* **5**:281.
3. Alam, J. (1990) *Anal. Biochem.* **188**:245-254.


## pTKβ

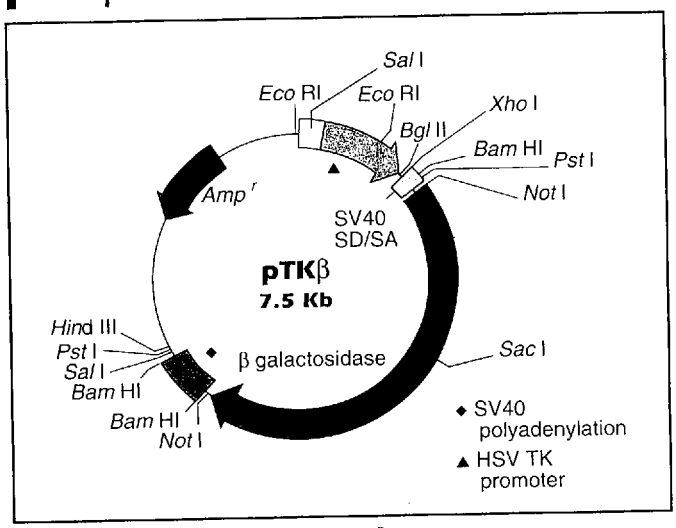



**Figure 12.34** Expression vector pTKβ

| Product | Size | Cat. # |
|---|---|---|
| pTKβ | 25 µg | 6179-1 |

### Description

pTKβ is a mammalian expression vector designed for the expression of β-galactosidase in mammalian cells as transcribed by the Thymidine Kinase promoter. pTKβ contains an RNA splice site, a polyadenylation signal, and the *E. coli* β-gal gene. The β-gal gene is located within a pair of *Not* I restriction sites used for easy excision and replacement with another gene of interest. pTKβ may be used for optimizing electroporation conditions, as a reference plasmid for reporter gene constructs, for "enhancer-trap" vectors, or for analyzing *cis* acting elements and trans acting factors in conjunction with an effective β-gal stain.

### Cloning site

*Not* I

### Vector Size

7.5 Kb

### References


1. MacGreggor & Caskey (1989) *Nucleic Acids Res.* **17**:2365.
2. Norton & Coffin (1985) *Mol. and Cell Biol.* **5**:281.
3. Alam, J. (1990) *Anal. Biochem.* **188**:245-254.

## CLONING VECTORS

# pUC18, pUC19

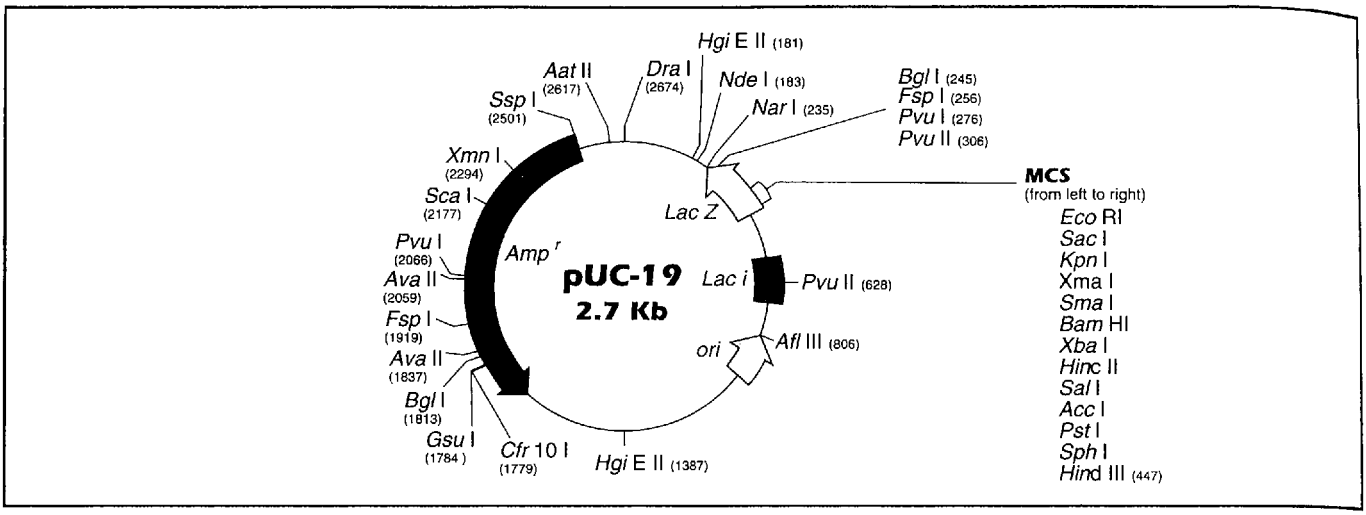



**Figure 12.35** Expression vector pUC19

12

### Description

pUC18 and pUC19 are prokaryotic cloning vectors sharing a multiple cloning site with more than 13 unique sites. This Multiple Cloning Site is located within the *lacZ* gene, resulting in the disruption of β-galactosidase activity by cloned inserts and the appearance of white colonies on X-gal + IPTG containing plates. The *E. coli amp* and *ori* elements allow for easy *E. coli* amplification and selection. pUC18 and pUC19 differ in the orientation of the Multiple Cloning Site.

### Unique cloning sites within the MCS region

*Acc* I, *Bam* HI, *Eco* RI, *Hinc* II, *Hind* III, *Kpn* I, *Pst* I, *Sac* I, *Sal* I, *Sma* I, *Sph* I, *Xba* I, *Xma* I

| Vector Size | 2.7 Kb |
|---|---|

### References


1. Yanisch-Perron, C., *et al.* (1985)*Gene* **33**:103.
2. Norrander, J., *et al.* (1985) *Gene* **26**:101.


### MCS

396 447

*Hinc* II *Sal* I

*Eco* RI *Kpn* I *Bam* HI *Acc* I

GAA TTC GAG CTC GGT ACC CGG GGA TCC TCT AGA GTC GAC CTG CAG

*Sac* I *Xma* I *Sma* I *Xba* I *Pst* I

*Sph* I

GCA TGC AAG CTT GG

*Hind* III

| Product | Size | Cat. # |
|---|---|---|
| pUC18 | 20 µg | 6110-1 |
| pUC19 | 20 µg | 6111-1 |

# Lambda BlueMid™

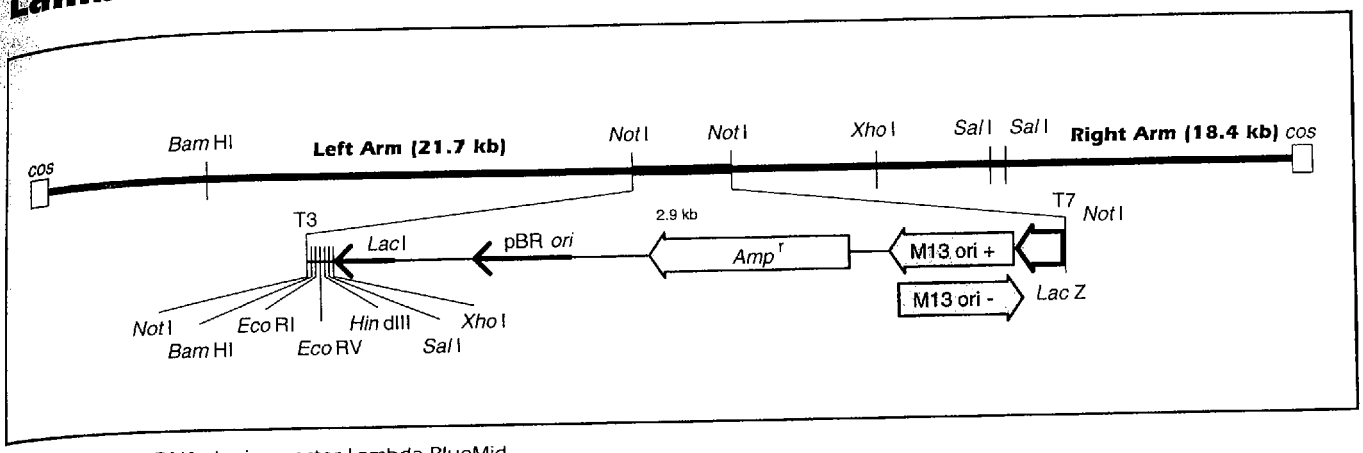



**Figure 12.36** cDNA cloning vector Lambda BlueMid

| Product | Size | Cat.# |
|---|---|---|
| Lambda BlueMid (+) | 10 µg | 6119-1 |
| Lambda BlueMid (-) | 10 µg | 6120-1 |

**Description**

cDNA cloning vectors for high-efficiency cloning with easy conversion to plasmid. Lambda BlueMid is designed with unique features that facilitate cloning large cDNAs (up to 7.0 Kb) and removal of complete inserts by converting them to plasmids. Thus, no subcloning or helper phage needed for plasmid conversion. Contains T3 and T7 promoters for in vitro transcription and bi-directional restriction mapping, and a M13 origin for easy single-stranded sequencing. Allows directional cloning into *Hind* III and *Eco* RI sites.

Lambda BlueMid is available as the (+) or (-) form, distinguished by the orientation of the M13 ori sequence after digestion with *Eco* RI and *Hind* III and dephosphorylation. A *Hind* III/*Eco* RI cloning linker/adaptor and complete protocol is provided.

**Unique cloning sites**

*Bam* HI, *Not* I, *Not* I, *Xho* I, *Sal* I

**Vector size** 43 Kb

# pWE15

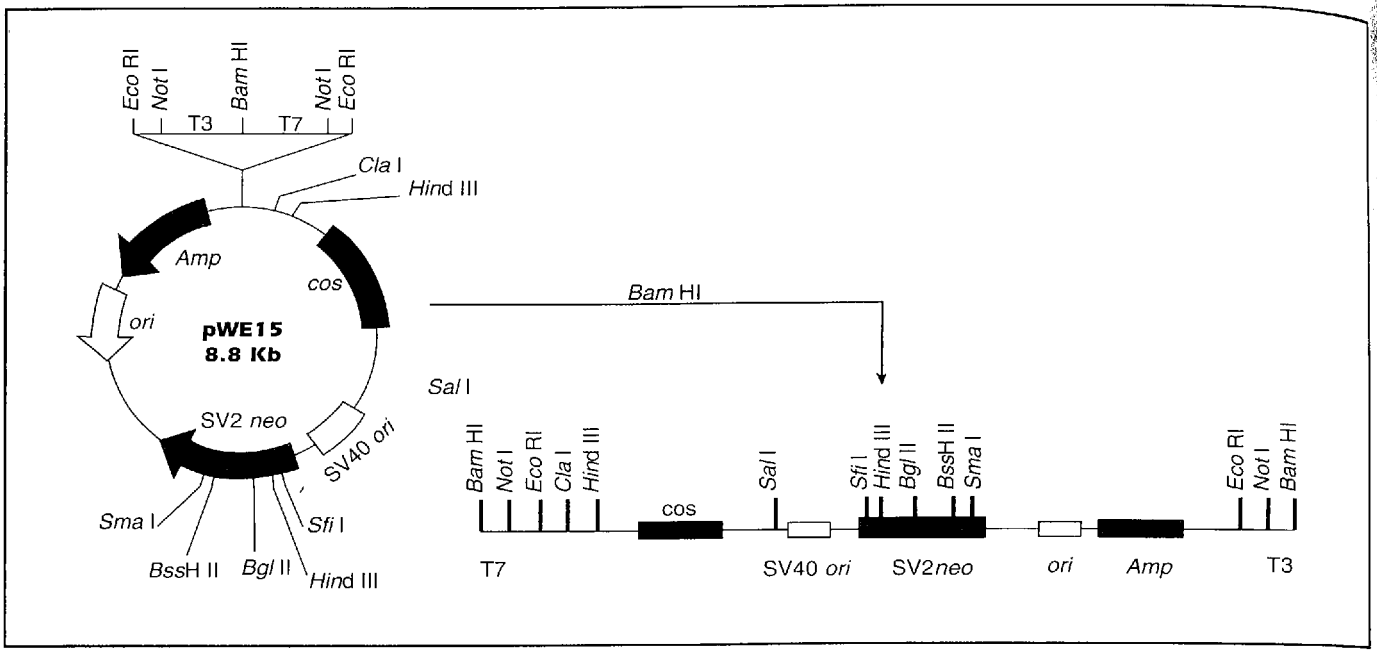



**Figure 12.37** Cosmid cloning vector pWE15

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| Product | Size | Cat. # |
|---|---|---|
| pWE15 | 20 µg | 6340-1 |
| | 100 µg | 6340-2 |

## Description

pWE15 is a cosmid cloning vector designed for rapid restriction mapping of genomic clones. This cloning vector permits the *in vitro* packaging of genomic DNA fragments in the 35-45 Kb range. Nested inside the 8 bp *Not* I sites are the T3 and T7 transcriptional promoters which flank the *Bam* HI cloning site to facilitate generation of end-specific RNA probes for the rapid identification of overlapping fragments (also known as chromosomal walking). Release of intact cloned insert DNA is accomplished by virtue of the rare-cutting *Not* I restriction enzyme flanking the *Bam* HI cloning site. pWE15 contains an SV40 promoter driving the neomycin selection marker for expression in eukaryotic cells. The presence of the ampicillin resistance gene also allows for prokaryotic selection.

## Unique cloning site

*Bam* HI

**Vector Size** 8.8 Kb

## References

1. Wahl, G., *et al.* (1987) *Proc. Natl. Acad. Sci. USA* **84**:2160.
2. Lau, Y. F. & Kan, Y. W. (1983) *Proc. Natl. Acad. Sci USA* **80**:5225.
3. Ish-Horowicz & Burke, J. F. (1981) *Nucleic Acids Res.* **9**:2989.

# EMBL 3, EMBL4

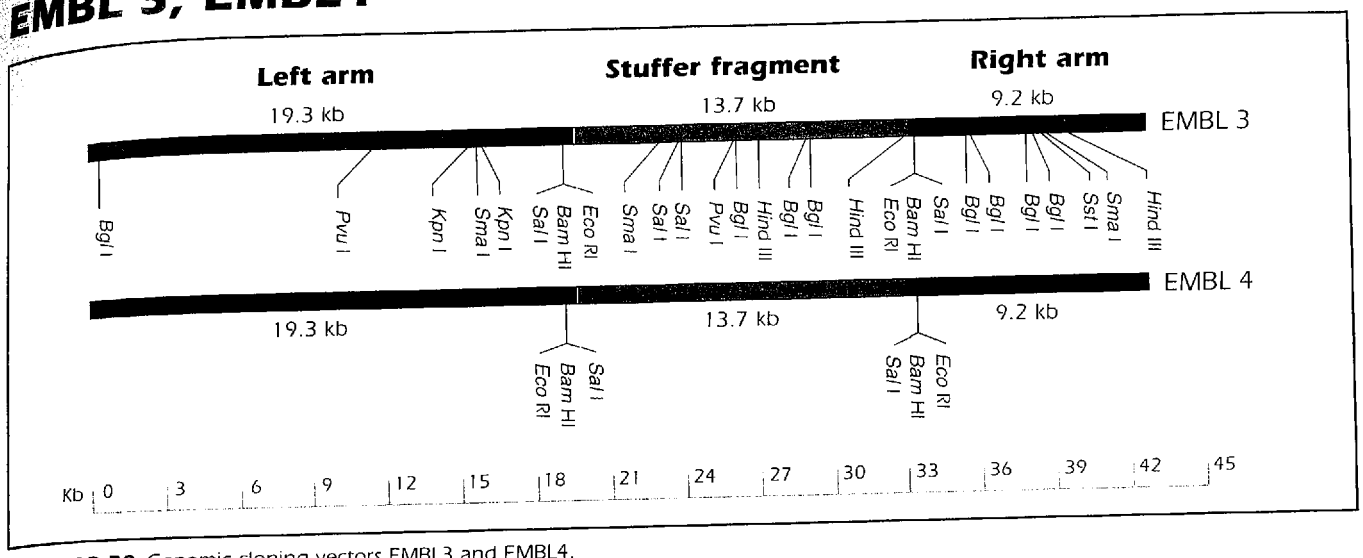


**Figure 12.38** Genomic cloning vectors EMBL3 and EMBL4.

## Description

EMBL 3 and 4 are high-capacity lambda replacement vectors which are particularly useful for the construction of genomic clone banks with partial *Mbo* I digests. Genomic cloning with lambda replacement vectors is more efficient for donor fragment recovery, and recombinant screening than is genomic cloning with cosmid vectors. These cloning vectors are suitable for cloning genomic DNA fragments in the 9.0-22.0 Kb range. Recombinants are easily selected by virtue of their Spi⁻ phenotype, which allows the phage to be amplified in *E. coli* strain K803 for EMBL-3, and K802 for EMBL-4.

EMBL3 and EMBL4 contain a 13.7 Kb "stuffer fragment" which is non-essential and is replaced by cloned insert DNA. A set of inverted polylinker sequences containing *Sal* I, *Bam* HI and *Eco* RI restriction sites are arranged at either end of the red+/gam+ stuffer fragment. The Multiple Cloning Sites are located between the lambda arms and the stuffer fragment so that double-digestion of the vector will prevent religation of the stuffer fragment to the vector arms. Therefore, when EMBL3 is digested with *both Bam* HI and *Eco* RI, or EMBL4 with *Bam* HI and *Sal* I it is impossible for a viable vector to religate without a foreign insert fragment present. As the *Bam* HI sites are usually not recreated, inserts can be removed from recombinants by digestion with either *Sal* I (EMBL3) or *Eco* RI (EMBL4) digestion. Recombinants are selected by their ability to grow on a P2 lysogen (K803 [EMBL-3], and K802 [EMBL-4]).

## Unique cloning site

*Bam* HI

## Vector Size

42.2 Kb

## References

1. Frischauf, A. M., *et al.* (1983) *J. Mol. Biol.* **170**:827.
2. Karn, J. M., *et al.* (1980) *Proc. Natl. Acad. Sci. USA* **77**:5172.

| Product | Size | Cat. # |
|---|---|---|
| EMBL3 (*Bam* HI Arms) | 10 µg<br>25 µg | 6108-1<br>6108-2 |
| EMBL4 (*Bam* HI Arms) | 10 µg<br>25 µg | 6109-1<br>6109-2 |

## Additional cDNA Cloning Tools

| Product | Size | Cat. # |
|---|---|---|
| Lambda DNA | 500 µg<br>2000 µg | 6250-1<br>6250-2 |
| Lambda gt10 DNA, uncut | 10 µg | 6332-1 |
| Lambda gt11 DNA, uncut | 10 µg | 6333-1 |
| Lambda gt Cloning Linker, *Eco* RI, dephosphorylated 12mer | 2.5 µg | 6490-1 |
| Lambda gt10 DNA, *Eco* RI cut | 10 µg | 6330-1 |
| Lambda gt11 DNA, *Eco* RI cut | 10 µg | 6331-1 |
| pBR322 DNA | 20 µg<br>100 µg | 6210-1<br>6210-2 |
| pBR322; dG-tailed, *Pst* I cut | 5 µg<br>25 µg | 6230-1<br>6230-2 |